IDENTIFICATION OF THE MOST AROMA-ACTIVE COMPOUNDS IN STRAWBERRIIES: VARIETY DIFFERENCES AND THE EFFECTS OF HEATING ON STRAWBERRY PUREE

By

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This dissertation is dedicated to my parents, Helen and Herb Schulbach.

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The overall objectives of this project were to determine the volatiles in strawberries that are important to fresh fruit aroma or contribute to aroma changes during heating. Solid-phase microextraction was compared with two common methods of volatile isolation, batch solvent extraction and dynamic headspace, and was found to be more efficient and less variable than the other two traditional methods. Volatiles were extracted from strawberry puree and evaluated using gas chromatography/olfactometry. Of the compounds identified, the ten most aroma-active volatiles were: diacetyl, methyl butyrate, ethyl butyrate, methyl-2-methyl butyrate, hexanal, E-2 hexenal, 1-octen-3-one, 2,5-dimethyl-4-hydroxy-3(2H)-furanone, linalool, and γ-decalactone. However, many additional aroma-active volatiles could not be identified because of their extremely low concentration in strawberry puree.

A sensory panel was trained using descriptive analysis to evaluate differences between strawberry cultivars based on the aroma attributes: fruity, green, floral, caramel, peach, and strawberry flavor intensity. Significant differences between cultivars were found for all of the aroma descriptors. Correlations were made between sensory analysis, gas chromatography/ofactometry analysis, and the measured concentration of certain volatiles. The ratings for peach were highly correlated with the presence of two compounds with peach-like aroma, γ-decalactone and γ-dodecalactone. The ratings for fruity were weakly correlated with the sum of the esters: ethyl butyrate, methyl butyrate, methyl-2-methyl butyrate, ethyl hexanoate, and methyl hexanote. The other aroma descriptors were not well correlated with the aroma-active volatiles that could be measured in the strawberry puree.

Solid-phase microextraction coupled with gas chromatography/pulsed flame photometric detection was used to monitor sulfur volatiles during the heating of strawberry puree. Carbon disulfide, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, methyl thioacetate, and methyl thiobutyrate were identified and quantified in the strawberry puree. Sulfur dioxide, hydrogen sulfide, methanethiol, and methional were also identified but could not be quantified. The compounds dimethyl trisulfide, methyl thiobutyrate, and methyl thioacetate, did not change significantly during heating, while dimethyl disulfide decreased by over 70%. The most important change was in dimethyl sulfide, which increased from an undetectable amount to a level over 100 times its aroma detection threshold.

CHAPTER 1
INTRODUCTION

Strawberries are produced throughout the world and are prized for their unique flavor. Florida is the second leading producer of strawberries in the United States and in 2000-2001 approximately 6500 acres were harvested with a total value of $167,310,000 (Florida Agricultural Statistics Service, 2002). The center of production is in the Plant City area East of Tampa Bay, and fruit is harvested from late November to March (Hancock, 1999).

In addition to fresh fruit, strawberries are widely used in juices, confections, pastries, and frozen desserts. Because of the fruit's popularity, the aroma of strawberry has been widely studied since the 1930s, and to date over 360 volatiles have been identified in the fresh fruit (Latrasse, 1991). However, this extensive list of volatiles has not produced an adequate understanding of the characteristic aroma of strawberry. More recently, the development of gas chromatography/olfactometry methods has begun to produce a better understanding of which volatile compounds have aroma activity, and which of them are the most responsible for the unique strawberry aroma. Differences in varieties, fruit maturity, storage conditions, sample preparation, volatile extraction techniques, and chromatographic methods have produced widely differing reports as to which volatiles are the "character impact" compounds in strawberries.

The most common strawberry, *Fragaria* x *ananassa*, is a hybrid of *Fragaria chiloensis* (L.) Duch. (a native of eastern North America) and *Fragaria Virginiana* Duch., which originated in western South America (Hancock, 1999). The two species

were widely grown in Europe and the first naturally occurring hybrids of the species were described in 1766 by Antoine Nicholas Duchesne (Hancock, 1999).

Genetic Studies to Improve Strawberry Flavor

Formal strawberry breeding began in England in 1817 by Thomas A. Knight who used clones of both species in his crosses (Hancock, 1999). Since those early breeding efforts, genetic improvement has focused on pest resistance, climatic adaptation, flowering and fruiting habit, yield, and fruit quality. Fruit quality considerations have generally ignored aroma and have instead focused on color, size, firmness, shape, soluble solids content, and acidity. The difficulty of describing and measuring fruit aroma and determining which compounds are responsible for that aroma has contributed to the neglect of this quality parameter. The exclusion of aroma as a quality parameter may be one reason that some modern varieties have been criticized for lack of full flavor.

More attention is now being paid to improving the flavor of fresh fruit and preserving the "fresh strawberry" flavor in processed products. Recently, Ulrich et al. (1997) profiled a large number of strawberry varieties using chemical analyses and grouped them into three major aroma types for breeding purposes. Kerler et al. (2000) went a step further and made crosses of eight strawberry cultivars and analyzed the fruit of nearly 4500 offspring for two important volatiles: γ-decalactone and DHF (2,5-dimethy-4-hydroxy-3(2H)-furanone). The authors demonstrated a 6 to 7 fold increase in the concentration of these selected volatiles in some of the daughter plants. Enzymes involved in synthesis of key aroma-active compounds have also been studied by a number of authors (Perez et al., 1999; Mitchell and Jelenkovic, 1995). Manning (1998) isolated ripening-related genes from strawberry for the purpose of determining their

function. These studies clearly indicate that there is a growing understanding of aroma-active compounds which may be used to develop improved cultivars of strawberries.

Sensory Studies of Strawberry Flavor

Sensory analyses using discrimination tests and descriptive analysis have been used for strawberry flavor analysis. Discrimination tests are used to determine if there is a noticeable difference between samples and are often used in processing or storage studies (Paakkonen and Mattila, 1991; Siegmund et al., 2001). Descriptive analysis involves the detection and description of quantitative and qualitative aspects of a product using trained panelists (Meilgaard et al., 1999). Ulrich et al. (1997) and Sims et al. (1997) used descriptive analysis to differentiate between varieties of strawberries. However, sensory analysis alone does not provide specific information on what makes two strawberry varieties taste different or what causes the change in aroma of strawberry juice during storage.

Chemical Analysis of Strawberry Fruit

One of the most important steps in chemical analysis of aroma volatiles is the extraction of these compounds from the food matrix. There is no ideal method for extracting aromas from foods, and obtaining a representative sample is difficult. Aroma-active compounds span a wide range of chemical classes, including acids, aldehydes, alcohols, carbonyls, and sulfur compounds. Many of these compounds are chemically unstable and may be lost during extraction and concentration, or artifacts may be formed. Many methods such as supercritical fluid extraction (Polesello et al., 1993), static headspace (Hamilton-Kemp et al., 1996), dynamic headspace (Miszczak et al., 1995), vacuum distillation (Schreier, 1980), and liquid-liquid extraction (Pyysalo et al., 1979) have been used to isolate compounds from strawberries. Although there is still no ideal

method for extracting volatile compounds from foods, the development of solid-phase microextraction (SPME) has greatly simplified the procedure. This method uses a fused silica fiber coated with an absorbent material to extract and concentrate aroma compounds. SPME is a rapid, low cost, solventless technique that facilitates the analysis of large numbers of samples and has been used by a number of authors to study strawberry aroma (Siegmund et al., 2001; Song et al., 1998; Ulrich et al., 1997). Once the compounds have been extracted, they are most commonly analyzed using gas chromatography. A flame ionization detector is the most commonly used detector, but a mass spectrophotometer is also commonly used as well as element specific detectors such as a pulsed-flame photometric detector.

Sensory tests and chemical analysis can then be correlated with each other to determine what changes in chemical composition are associated with aroma change. Multivariate statistical techniques such as principal component analysis (PCA), partial least squares (PLS) or multivariate analysis of variance (MANOVA) are commonly used to determine these relationships. However, it is important to note that correlations do not imply cause. For example, Krumbein and Auerswand (2000) found a very high correlation (R= 0.73) between the sensory descriptor "fruity" and the compound 1-octen-3-one. The correlation does not indicate if this compound (which has a strong mushroom aroma) contributes to fruity aroma or if its concentration simply changes in conjunction with other compounds that actually produce the fruity aroma.

Gas Chromatography/Olfactometry

Instead of simply correlating chemical changes with sensory analysis, chemical and sensory analyses can be combined using a hybrid analytical/sensory methodology called gas chromatography/olfactometry (GC/O). In this technique, volatile compounds from a sample are volatilized, separated in the gas chromatograph, and sent to a sniff port where a human subject is used as a detector. This direct link between chemical and sensory analysis provides a means for determining which volatiles in the sample have aroma activity. As mentioned previously, the value of the analysis is only as good as the quality of the extract. The aroma activity of a volatile compound that has been volatilized at high temperature in the injector of a gas chromatograph may not be a good representation of the aroma activity of the compound at room temperature in the original sample matrix. Additionally, the sensitivity of the limited number of subjects analyzing samples may not reflect the same sensitivities as the public at large. In spite of these limitations, GC/O is a very useful tool and has been used for aroma profiling of strawberry fruit (da Silva and das Neves, 1999), to study heat-induced changes in strawberry juice (Schieberle, 1994) and jam (Lesschaeve et al., 1991), and to study changes in a strawberry beverage during storage (Siegmund et al., 2001).

Strawberry Aroma

At least 131 esters have been reported in strawberries and they largely dominate the total volatiles both qualitatively and quantitatively (Latrasse, 1991). Methyl butyrate, ethyl butyrate, ethyl–2-methyl butyrate, methyl hexanoate, and hexyl and E-2-hexenyl acetates have been suggested as the most important esters in strawberries (Latrasse, 1991; Schieberle and Hofmann, 1997). These compounds have generally been described as having "fruity" character. The compound DHF (2,5-dimethy-4-hydroxy-3(2H)-furanone)

has often been reported to be the most important volatile in strawberry (Schieberle and Hofmann, 1997; Ulrich et al., 1997). It has an aroma often described as caramel or cotton candy. The most important compounds that give a "green" note have been reported to be hexanal, Z-3-hexenal, and Z-3-hexen-1-ol (Ulrich et al., 1997; Latrasse, 1991). Dirinck et al. (1981) have shown that the sulfur compounds methanethiol and dimethyl sulfide are important to the aroma of certain varieties. Other important compounds that may contribute to fruity and floral aroma are linalool, γ-decalactone, and methyl anthranilate.

Strawberry flavor and aroma have been shown to change during processing and storage. A significant portion of the strawberry crop in Florida is not suitable for fresh market due to visual defects, but this fruit could be processed (Golaszewski et al., 1998). Studies on the flavor changes due to freezing (Douillard and Guichard, 1990; Deng et al., 1996), high-pressure treatment (Lambert et al., 1999), heating (Lesschaeve et al., 1991; Sloan, 1969), and storage of juice (Siegmund et al., 2001) have been published.

Although many studies on strawberry flavor and aroma have been performed, the chemical basis of strawberry aroma remains to be elucidated. The difficulty of the integration of sensory tests and chemical analysis is one of the main reasons. However, strawberry fruit and strawberry flavored products are extremely popular, and studies to improve the aroma quality of fresh fruit and processed products have provided continual improvement in the flavor and acceptance of this popular fruit.

Objectives

The overall objectives of this project were to determine the volatiles in strawberries that are important to fresh fruit aroma or contribute to aroma changes during

heating. The first task was to identify a suitable extraction method to use with gas chromatography for rapid screening of strawberry cultivars or for monitoring volatile compounds during processing of strawberry juice or puree. The second task was to determine the volatile compounds that are most important to the aroma of fresh strawberry fruit through the correlation of sensory analysis with chemical analyses. The final task was to determine the major sulfur volatiles in strawberries, to quantify their changes during heating, and to estimate the sensory impact of the changes in concentration of these sulfur compounds.

CHAPTER 2
LITERATURE REVIEW

Strawberry Aroma

Strawberry aroma can be described as a mixture of fruity, floral, caramel, jam, and green notes. The aroma is very complex and more than 360 volatile compounds have been identified in the fresh fruit (Latrasse, 1991). While it is likely that only a handful of these compounds comprise the basis of strawberry aroma, many additional compounds are needed to produce the well-rounded aroma of a fresh strawberry. The aroma compounds of strawberry include organic acids, alcohols, carbonyls, esters, furans, sulfur compounds and others (Hong et al., 1990) which make the isolation of a representative sample of volatiles difficult.

While many authors have reported on the chemical analysis of strawberry fruit, these reports were often not combined with sensory impact studies. Hirvi et al. (1983) was among the first to attempt to determine the importance of the various volatile compounds to the overall flavor of the fruit. In this study, ethyl hexanoate, ethyl butanoate, trans-2-hexenal, mesifuran and linalool were determined to be the most important aroma compounds even though ethyl hexanoate and linalool were not found in all varieties. Ulrich et al. (1997) reported that methyl anthranilate is a very important character impact compound in certain strawberry varieties and divided strawberry varieties into high and low methyl anthranilate types. Using discriminant analysis, the authors further divided the low methyl anthranilate types into two groups, the ester types and the DHF (2,5-dimethy-4-hydroxy-3(2H)-funanone) types. Both DHF and mesifuran

(2,5-dimethy-4-methoxy3(2H)-funanone) were determined to be important aroma impact compounds in all three of these groups. Other important aroma impact compounds identified by these authors are butanoic acid, 2-methyl butanoic acid, hexanoic acid and γ-decalactone.

Recently, a few studies using gas chromatography/olfactometry (GC/O) methods to characterize the aromas of strawberries have been published. Da Silva et al. (1999) used purge-and-trap with GC/O to assess the aromatic properties of 40 individual components of strawberry fruit. In a study to evaluate the effect of cooking on volatiles of strawberry jam, Lesschaeve et al. (1991) used GC/O to describe the aroma profile of strawberries heated using two different methods. Lambert et al. (1999) studied the effect of high-pressure treatment on the flavor of fresh strawberry puree. Although they did not use GC/O, they reported a fairly complete aroma profile for their samples using gas chromatography/mass spectrometry. Schieberle (1994) also studied the heat-induced changes in strawberry volatiles using aroma extract dilution analysis (AEDA). Ulrich et al. (1997) used Freon extraction and GC/O to characterize the flavor of a number of strawberry varieties. Ulrich et al. (1997) also suggested using solid-phase microextraction (SPME) as a simple extraction method for characterizing aromas of strawberries for use in breeding programs.

Of the 360 or so compounds listed for strawberries, it is not clear which are the most important. Table 2.1 provides a summary of the compounds that have been reported in strawberry along with an accompanying aroma descriptor. This shorter list, developed mostly from GC/O analysis, probably contains most of the important volatiles that make up strawberry flavor. The compound DHF has often been reported to be the most

Table 2.1. Aroma-Active Compounds Reported in Strawberries With Their Associated Descriptors.

Compound	Aroma
acetic acid	sour[a]
2,3-butanedione	buttery[a]
butanoic acid	sweaty, rancid[ab], cheesy[c]
3-hydroxy-2-butanone	green pepper[c]
butyl acetate	acetone[b]
cinnamyl acetate	fruity[c]
γ-decalactone	fruity, lactone-like[b]
δ-decalactone	lactone-like[b]
γ-dodecalactone	peach, apricot[c]
decanoic acid	grassy[c]
dodecanoic acid	fruity[c]
ethyl butyrate	fruity[ab]
ethyl hexanoate	green apple[b] slightly acid[c] fruity[d]
ethyl 2-methylbutanoate	fruity[ad]
ethyl 2-methylpropanoate	fruity[ad]
eugenol	spicy, nutmeg[b]
2-furaldehyde	baked potatoes[c]
DHF	caramel-like[ad] strawberry jam[c]
heptanone, heptan-2-one	meaty[b]
hexanoic acid	unpleasant[b]
Z-3-hexenal	green, leaflike[ab]
E-2-hexenal	green, fatty[b]
(E or Z) 2-hexen-1-ol	grassy[b] green[d]
β-ionone	floral, violet[b]
linalool	flowery, sweet[b] citrus[d]
mesifuran	sweet[d] smoky[a] floral[c]
methional	cooked potato[d]
methyl anthranilate	aromatic, sweet, soap[b]
methyl butyrate	fruity[abd]
methyl 2-methy butyrate	fruity[a]
2-methylbutanoic acid	sweaty[ad] fruity, buttery[b], cheesy[c]
2-methylpropanoic acid	floral[c]
4-methylpentanoic acid	floral[c]
methyl hexanoate	fruity/pineapple[b]
myrcene	spicy[d]
E-nerolidol, nerolidol	floral[c]
nonanal	floral[c]
E-2-nonenal	fruity[c]
1-octen-3-one	mushroom[d]
octanoic acid	unpleasant[b] grassy[c]
pentanone-2	acetone[b]

[a] (Schieberle and Hofmann, 1997), [b] (Ulrich et al., 1997), [c] (Lesschaeve et al., 1991), [d] (Siegmund et al., 2001)

important volatile in strawberry (Schieberle and Hofmann, 1997; Ulrich et al., 1997). It has an aroma often described as caramel or cotton candy. At least 131 esters have been reported and they largely dominate the total volatiles in strawberry both qualitatively and quantitatively (Latrasse, 1991).

Methyl butyrate, ethyl butyrate, ethyl-2-methyl butyrate, methyl hexanoate, and hexyl and E-2-hexenyl acetates have been reported as the most important esters in strawberries (Latrasse, 1991; Schieberle and Hofmann, 1997). These compounds have generally been described as having "fruity" character. The most important compounds that give a "green" note have been reported to be hexanal, Z-3 hexenal, and Z-3-hexen-1-ol. (Ulrich et al., 1997; Latrasse, 1991). Other important compounds that may contribute to fruity and floral aroma are linalool, γ-decalactone, and methyl anthranilate.

Processing and Storage Effects

Larsen and Poll (1995) studied the effects of freezing and thawing on the changes in aromatic compounds in strawberries. They found decreases in E-2-hexenal and hexanol and increases in volatile acids, linalool, methyl hexanoate, ethyl butyrate and ethyl hexanoate. Conversely, Douillard et al. (1990) reported decreased levels of low-boiling esters such as ethyl butyrate in frozen berries. Larsen and Poll (1995) suggested that the different results were due to the fact that they analyzed thawed berries while the other authors began analysis while the fruit was still frozen. Deng et al. (1996) attributed off-flavor in frozen-thawed strawberries to an increase in hydrogen sulfide.

Heating also results in large changes in the volatile profile of strawberry. Schieberle (1994) found a significant reduction in fruity notes (5 esters) and green notes (Z-3 hexenal), while nine new compounds appeared as the result of heating. The author

also noted a near doubling of DHF in the heated juice resulting in an increase in the sweet caramel-like note. Sloan et al. (1969) detected increases in dimethyl sulfide, aceteldehyde, isobutyraldehylde, furan, 2-furaldehyde, 2-acetyl furan, and ethyl furoate in strawberry puree heated at 120°C for 30 min.

Changes in the aromatic volatile composition of strawberry after high-pressure treatment has also been studied. Lambert et al. (1999) found no major alteration in the strawberry aromatic profile when processed at 200 or 500 MPa for 20 minutes. At 800 MPa, new compounds were detected and the relative concentration of the remaining substances changed significantly. The 800 MPa treatment was also significantly different from normally heated strawberry samples, where compounds such as geraniol, vanillin, and other unidentified compounds were detected.

Aroma changes considerably when fruit homogenized. This process disrupts the cell structure, releasing enzymes that alter the flavor profile of the fruit. However, the full extent of this flavor change has not been described for strawberries. One of the major pathways for volatile production resulting from cellular disruption involves enzymatic lipid oxidation. Lipoxygenase and hydroperoxide lyase activity result in the production of C-6 aldehydes (hexanal from linoleic and Z-3-hexenal from linolenic acid). These aldehydes then produce hexanoic acid and the corresponding alcohols and esters. Pentanoic acid (and esters) and butanoic acid (and esters) have also been shown to result from α-oxidation and β-oxidation of hexanoic acid in apples (Rowan et al., 1999).

Riley and Thompson (1998) studied the generation of aldehydes following tissue disruption in tomato fruit. They compared homogenized tomato fruit with fruit homogenized in buffer with saturated calcium chloride to inhibit enzyme activity. The

fruit homogenized in buffer (pH 7) showed minimal endogenous aldehyde content. However, volatile levels increased up to 10-fold following homogenization of the ripe tomato fruit without the salt.

Enzymes involved in these pathways have been described in a number of fruits. Mitchell and Jelenkovic (1995) described NAD-dependent and NADP-dependent alcohol dehydrogenase enzymes of strawberries. NAD-dependent activities were greatest against short-chained alcohols, whereas the NADP-dependent activities were most active against aromatic and terpene alcohols. Perez et al. (1993) purified and conducted kinetic studies on alcohol acyltransferase of strawberry fruit. Maximum activity was observed using acetyl-CoA and hexyl alcohol as substrates. A clear correlation was seen between the strawberry ester profile and enzyme preference. Perez et al. (1999) studied the lipoxygenase and hydroperoxide lyase activities of ripening strawberry fruit. Linolenic acid was the preferred substrate for lipoxygenase, forming 13-hydroperoxides and 9-hydroperoxides in the proportion 70:30. The hydroperoxide lyase was found to then cleave the 13-hydroperoxide to Z-3-hexenal (hexanal from linoleic acid).

Chemical Analysis of Fruit Volatiles

One of the most important steps in flavor analysis is the extraction of the aroma-active compounds from the food matrix. There are many factors making the isolation difficult. The food matrix presents many problems due to the content of many reactive substances such as sugars, proteins, lipids and vitamins. In fact, since many volatile compounds arise from these reactions, differences in handling of the product during extraction will produce differences in the final extract. The presence of emulsifiers such as proteins and lipids in foods can result in emulsion formation that can trap volatiles and complicate some extraction methods. Volatile compounds span a wide range of polarity,

but so do many non-volatile compounds. The extraction of lipids, vitamins, carotenoids and other compounds by non-polar solvents interferes with the isolation of many flavor compounds. Water-soluble compounds such as sugars or proteins also complicate the isolation of more polar compounds. Water also causes problems in distillation methods due to its relatively high volatility and high concentration in many food products. Another source of difficulty is that foods also contain large amounts of volatile compounds without aroma activity. These isolation problems are made worse by the fact that many aroma compounds have significant aroma at concentrations in the parts per billion range or lower. For example, DHF, an important aroma compound in strawberries, has been reported to have an aroma threshold (in water) of 40 parts per billion. Aroma compounds are found in a wide range of chemical classes and no single isolation method can uniformly extract them all (Reineccius, 1994).

The first step in chemical analysis is to extract the aroma volatiles from the fruit. Volatiles can be isolated from headspace and in this case, isolations from intact fruit can be made. More commonly, the sample is prepared by crushing or blending the fruit. However, the cellular disruption activates enzymes that can quickly alter the aroma profile. Additionally, microbial processes such as fermentation may take place if the isolation step is too long (Reineccius, 1994). One common method to prevent these problems is to homogenize the fruit with methanol. One disadvantage of this step is that the methanol may interfere with further isolation steps. An alternative is to blend the sample with a concentrated solution of salts such as NaCl or $CaCl_2$ (Buttery et al., 1987). The salt inactivates the enzymes and microorganisms and has the additional advantage that it can aid in the isolation of the volatile compounds. Solvent extraction, dynamic

headspace, and solid-phase microextraction (SPME) are commonly used methods for isolation of volatiles from the sample. Since it has been estimated that sampling, collecting, and preparation steps such as extraction, concentration, fractionation, and isolation can take up to 80% of the total analysis time (Kataoka et al., 2000), selection of an analytical method is very important. Differences in the published reports on the most important compounds in strawberries can be due to cultivar, season, ripeness, or storage factors, however, many of the differences are likely due to the difficulties in isolating a representative sample.

Solvent Extraction

Most aroma compounds are soluble in organic solvents. Lipids are also highly soluble and interfere with the extraction. However, the lipid content in strawberries is too low to cause a large problem. The simplest form of solvent extraction is batch extraction. The sample is mixed with an immiscible solvent and the volatiles partition between the solvent and the aqueous phase. Because the solvent is immiscible with water, it will separate from the sample solution where it can be siphoned off. The extraction efficiency of this method is based on the partition coefficient and the relative amounts of solvent and sample. Repeated extraction is much more efficient than a single extraction and the amount of analyte extracted can be calculated from the following formula.

$$W_r = W_o (V_w/(DV_o + V_w))^N$$

Where W_r = weight of solute remaining following extraction, W_o = weight of solute in original solution, V_w = volume of aqueous phase, V_o = volume of extracting solvent, D = partition coefficient, and N = number of extractions.

Solvents with high partition coefficients such as dichloromethane or chloro-floro-carbons such as Freon 113 are commonly used and give high recovery rates (Table 2.2).

However, with multiple extractions, many other solvent systems such as pentane/ether or ethyl acetate work well. Ferreira et al. (1998) evaluated solvent systems such as

Table 2.2. Recovery of Model Compounds From an Alcohol-Water System.

	Solvents, Recovery (%)[a]			
Compound	Freon I	DCM[b]	Ether	Isopentane
Ethyl Butyrate	66	43	--	16
2-Methyl-1-Propanol	34	55	22	32
3-Methyl-1-Butanol	63	66	50	48
1-Hexanol	85	67	23	38
Benzaldehyde	83	54	18	20
Acetophenone	53	41	34	20
Benzyl formate	75	56	21	25
Methyl anthranilate	62	59	57	27

[a] Batch extraction of model system extracted 6 x 50 mL solvent.[b] Dichloromethane.
Source. Cobb and Bursey(1978).

dichloromethane, Freon 113, pentane/diethyl ether and ethyl acetate/pentane for the analysis of wine using a model 12% hydroalcoholic solution (Table 2.3). Overall, dichloromethane had the highest partition coefficients followed by Freon 113. However, when 3.3 g of $(NH_4)_2SO_4$ was added to the 7.9 g sample, the extraction efficiency of other solvent systems such as pentane/diethyl ether (1:1) or ethyl acetate/pentane (1:3) improved to where they were in the same range as dichloromethane. The addition of salt increased the extraction efficiency of the Freon 113, but the partition coefficients of the analytes for this solvent had a much wider range, indicating that Freon 113 would not produce as representative of a sample as the other solvent systems.

Schieberle (1994) evaluated heat-induced changes in strawberry by extracting the volatiles 3 times with ethyl ether. He used 600 g of strawberries homogenized with 600 g saturated $CaCl_2$. The homogenate was centrifuged and 200 g of supernatant was

Table 2.3. Partition Coefficients of Selected Analytes Between Solvent Systems and a 12% Hydroalcoholic Solution and the Same Solution with a Salting-Out Effect.

Analyte	Freon 11		DCM		P/DE		EA/P	
	normal	w/salt	normal	w/salt	normal	w/salt	normal	w/salt
Ethyl Pentanoate	87	289	47	86	11	45	17	86
Linalool	42	204	94	130	12	58	18	99
Ethyl Decanoate	21	51	24	61	12	32	18	45
Isovaleric Acid	0.3	19	3	120	1	91	0.5	60
β-Ionone	57	15	44	38	12	14	19	22
γ-Nonalactone	22	85	161	119	6	68	8	75
Euganol	23	64	161	107	9	65	11	63

DCM = dichloromethane, P/DE = pentane/diethyl ether, EA/P = ethyl acetate/pentane.
Source. Ferreira et al. (1998).

extracted 3 times with diethyl ether (total volume 1.0 L) and the volatiles and the solvent were isolated by high-vacuum sublimation and concentrated to 300 μL. Siegmund et al. (2001) extracted volatiles from 200 mL of strawberry drink using a single extraction with 20 mL flurotrichlormethane. The organic extract was concentrated using a rotary evaporator to a volume of 500 μL. Lambert et al. (1999) used liquid/liquid extraction to measure volatiles in strawberry puree after ultra high-pressure treatment. Approximately 150 g of puree was extracted 3 times with 50 mL of dichloromethane and concentrated to a final volume of 500 μL, first using a Kuderna-Danish apparatus and then a stream of nitrogen.

Dynamic Headspace

Measuring headspace volatiles is an attractive analytical method because the aroma-active compounds are in a concentration similar to what they would be when the food is smelled. A sample of the headspace above a sample can be used directly but many volatiles are often in too low of a concentration to be quantified using most common detectors. For this reason, volatiles in the headspace are often concentrated using dynamic headspace or purge-and-trap methods. Typically the sample is placed in a

closed container and a flow of inert gas such as nitrogen is used to purge the headspace onto an adsorbent or a cryogenic trap. Cryogenic traps are more complex than adsorbent traps but they are the least selective and will collect virtually any aroma compound in the sample (Reineccius, 1993). However, cryogenic traps will also concentrate water (the most abundant volatile in food), and the aqueous sample must be further processed.

Common adsorbents, on the other hand, do not trap much water, and the traps can be thermally desorbed directly into the injector of a gas chromatograph. Tenax is one of the most common trapping materials because its high thermal stability makes it the best choice for thermal desorption (Nunez et al., 1984). Tenax traps can be easily conditioned by holding them at 260°C for 24 hr while purging with helium at 12 mL/min (Buckholz et al., 1980). Adsorbed volatiles are stable in the Tenax traps for up to 5 days at 0°C with no decomposition of adsorbed volatiles (Buckholz et al., 1980). However, Tenax has a low adsorption capacity and a low affinity for polar and small aroma-active molecules such as hydrogen sulfide (Reineccius, 1993).

There are many other adsorbant materials used for pre-concentration of volatiles, and no single adsorbant is best for all applications (Nunez et al., 1984). Many materials do not have sufficient temperature stability to be used with thermal desorption, and for these materials, solvent desorption is often used (Table 2.4).

A tube packed with an adsorbant acts like a chromatographic column. As the carrier gas moves through the column, compounds will also move and eventually will breakthrough and elute from the column (Nunez et al., 1984). During the purge-and-trap procedure, compounds that are not strongly adsorbed will move more rapidly through the column than compounds that are more strongly adsorbed. Buckholz et al. (1980) placed

three Tenax traps in series to evaluate breakthrough. Many of the highly volatile compounds broke through the first and second traps while almost all of the less volatile compounds were retained in the first trap. In addition to breakthrough, there are many

Table 2.4. Physical Properties of Some Adsorbants Used for Preconcentration of Organic Volatiles from the Vapor Phase.

Compound	Surface area (m^2/g)	Mean pore Dia.(Å)	TemperatureLimit(°C)
Tenax GC	19-30	720	450
Chromosorb 102	300-400	90	250
Chromosorb 105	600-700	500	200
Porapak Q	630-840	75	250
XAD-4	750	50	200
XAD-7	458	80	150

Source. Sucan et al. (1998).

other factors that affect the representative nature of the sample extract. The retention of compounds is affected by the form and size of the trap, the amount and type of adsorbant, the flow rate of the stripping gas, the time of trapping, and the temperatures of the sample and of the trap (Nunez et al., 1984). Using dry pet food, Sucan et al. (1998) evaluated the effect of many of these parameters on a trap consisting of 100 mg of Tenax-TA. They evaluated sample temperatures from 50°C to 90°C and found that while the total volatiles increased as temperature increased, the proportion of low boiling point compounds increased at a higher rate than the high boiling point compounds. They also found that artifacts were a significant portion of the total aroma isolated at 90°C. Increasing the sample size increased the total amount of trapped volatiles, but the proportion of the low boilers increased relative to the high boilers. The authors also evaluated purge gas flow rates from 10 to 50 mL/min. A flow rate of 30 mL/min trapped the most volatiles, but at higher flow rates, the amount of high boilers continued to increase. Buckholz et al. (1980) also evaluated the effect of flow rates from 10 to 60 mL/min on the volatiles from

peanuts using Tenax traps. They evaluated the sensory quality of the extracts and found that a flow rate of 40 mL/min produced a sample that most resembled natural peanut aroma. Sucan et al. (1998) also evaluated the effects of sampling time. Longer sampling times resulted in greater amounts of trapped volatiles, but the amounts of low boilers decreased with times longer than 60 minutes. They attributed the decrease to either breakthrough or displacement by higher boiling compounds that may bind more tightly to the adsorbant. Buckholz et al. (1980) evaluated the effects of sampling time with similar results. In addition to comparing peak areas, the authors performed sensory analysis on the different samples. A team of 3 experienced flavorists judged that the 4-hour collection time produced an aroma most similar to true roasted peanut aroma. The evaluators found that harsh green notes predominated in the 2-hour collection time and that burnt notes predominated in the 12-hour collection time. It is clear from these studies that sampling parameters must be optimized in order to obtain a representative sample. In spite of these variables, purge-and-trap is an accurate method of quantifying volatiles.

Shamaila et al. (1992) used purge-and-trap to study volatiles from strawberries stored under modified atmosphere. Sliced strawberries (300g) were placed in a flask at 40°C and nitrogen was purged through the flask at 30 mL/min for two hours and volatiles were trapped in a glass tube containing 120 mg of Tenax GC. The trap was eluted with 2 mL diethyl ether and the extract was concentrated to about 200 μL with a gentle stream of nitrogen. Da Silva et al. (1999) extracted volatiles from 100 g of fresh strawberries held at 45°C for 2 hours onto 90 mg of Tenax GC using a flow of 45 mL/min of nitrogen. Volatiles from the trap were directly injected into the gas chromatograph using an

automatic purge-and-trap injector. Buckholz et al. (1980) found good reproducibility (CV=1.24%) and precision (CV=3.5%) using purge-and-trap for analysis of selected volatiles from peanuts.

Solid-Phase Microextraction

Solid-phase microextraction (SPME) is a relatively new technique developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990). Sampling devices (manual and autosampler) consist of a coated silica fiber inside a hollow needle. The needle protects the fiber during insertion through the septa of the GC injector or sample container. The sampling device has a spring-loaded plunger so the fiber can be pushed out of the needle for sampling or desorption, and then withdrawn back into the needle for protection from physical damage. Solid-phase microextraction was initially developed as a water sampling technique. The fiber was immersed in the water, and compounds were absorbed onto the fiber. The simplicity of this method prompted researchers to investigate its use for food analysis. However, immersion proved impractical for many food products since proteins and other non-volatile compounds permanently bond to the fiber, reducing its functionality.

Methods for sampling headspace were soon developed and offered an additional advantage that the equilibrium between the fiber and the headspace can be up to an order of magnitude faster than equilibrium between a liquid and the fiber (Ai, 1999). This is mostly due to the much slower diffusion of analytes in a liquid compared to diffusion in the gas phase (Pawliszyn, 1999). Yang and Peppard (1994) compared liquid emersion and headspace sampling for 25 common flavor compounds. In general, the earlier eluting compounds on a polar column were more efficiently extracted from the headspace and

later eluting compounds were more efficiently extracted by direct emersion. Headspace is by far the most common method of SPME analysis of foodstuffs.

As SPME became more popular, new types of fiber coatings were developed and expanded the potential applications. The fiber coatings that are currently available differ by their polarity and whether they are adsorbent or absorbent (Table 2.5).

Table 2.5. Some Common Fiber Coatings Used with SPME.

Coating Material	Uses	Type
PDMS	Nonpolar semivolatiles (MW 60-275)	Absorbent, Nonpolar
Polyacrylate	Polar semivolatiles (MW 80-300)	Absorbent, Polar
PDMS/Carboxen	Trace-level volatiles (MW 30-225)	Adsorbent, Bipolar
PDMS/DVB	Volatiles, nitrogen compounds (MW 50-300)	Adsorbent, Bipolar
CW/DVB	Alcohols and polar compounds (MW 40-275)	Adsorbent, Polar
DVB/Carb./PDMS	Volatiles and semivolatiles (MW 40-275)	Adsorbent, Bipolar

PDMS = Polydimethylsiloxane, DVB = Divinylbenzene, CW = Carbowax.
Source. Sigma-Aldrich (1998).

The absorbant type coatings, polydimethylsiloxane (PDMS) and polyacrylate (PA), were the first commercially available fibers. Absorbent fibers extract analytes by partitioning them into a liquid-like phase. Analytes migrate in and out of the coating depending on their partition coefficient between the fiber and its environment. As long as the partition coefficient is determined by the activity coefficient of the analyte in water, and this coefficient does not depend on the presence of other organic molecules in water, absorption is a non-competitive process (Gorecki, 1999). This means that the partition of an analyte between the sample and the fiber is not influenced by other organic molecules as long as the concentrations are low.

Adsorbent coatings extract analytes by physical interaction. The adsorbent material is typically a solid material containing pores in which the analytes can be trapped temporarily. Polymer coatings such as polydimethylsiloxane/divinylbenzene

(PDMS/DVB) and PDMS/carboxen extract compounds by adsorption. Molecules adhere to the coating by Van der Waals, dipole-dipole, or other weak molecular forces (Gorecki, 1999). There are only a limited number of surface sites for adsorption and there is competition among molecules for these sites. This means that the amount of analyte extracted can be affected by other molecular species. A molecule with a high affinity for the surface can displace a molecule of lower affinity. Care must be taken when using adsorptive coatings because the linear range of analyte uptake is much less than for absorption and the dependence is non-linear. This makes quantitative analysis more difficult. However, adsorptive SPME is very popular and has been used successfully in many studies.

Initially, SPME was considered an equilibrium technique where the concentration of the analyte was estimated by calculating partition coefficients between the phases. When the sample matrix is simple, the distribution constants are very similar to those in a pure matrix and calibration might not be necessary since the distribution constants for calculating the sample concentration are available in the literature or can be calculated from chromatographic retention indicies.

However, Ai (1999) has shown that quantification is possible before equilibrium is reached. His results show that under non-equilibrium conditions the amount of extracted analyte is proportional to its concentration in the sample matrix as long as the sampling conditions are carefully controlled.

Under non-equilibrium conditions, calibration is necessary. External calibration curves can only be used if the standards are made up in the same matrix as the test sample. For complex samples such as foods, internal calibrations such as isotopic

dilution or standard addition should be used. Care must be taken to ensure that the response is linear in the concentration range of the sample and the spiked sample, and multiple standard addition is advised whenever practical (Ai, 1999).

The selection of the fiber coating is the most important step governing the amount of analyte extracted (Li and Weber, 1999). There are currently several kinds of coatings commercially available. The effect of fiber polarity can be seen in Table 2.6. PDMS/carboxen is bipolar and has a very high capacity, especially for relatively small molecules.

Table 2.6. Effects of SPME Fiber Coating on Analyte Extraction.

	Relative Detector Response				
Analyte	PDMS	PA	PDMS/DVB	CW/DVB	PDMS/CAR
Methanol	0	170	30	75	630
Ethanol	35	180	110	130	5250
Acetonitrile	140	230	160	130	6500
Isopropanol	180	360	600	250	97700
N-propanol	220	1200	1200	450	53400
Acetone	400	260	640	250	83000
Ethyl acetate	1500	2700	14000	4700	449500
2-methyl-3-propanone	4000	2100	48000	13000	821000

PDMS = polydimethylsiloxane, PA = polyacrylate, DVB = divinylbenzene, CW = carbowax, CAR = carboxen.
Source. Sigma-Aldrich (1998).

In addition to the fiber type, the amount of analytes transferred from a sample to the SPME fiber is highly dependant on the sample matrix. The addition of a salt such as sodium chloride can greatly affect the efficiency of extraction. Using a PDMS coated fiber, Yang and Peppard (1994) analyzed the effect of increasing salt concentrations on individual flavor compounds in an aqueous solution. The authors found that for some compounds, absorption increased, for some compounds absorption increased and then leveled off, for some compounds absorption initially increased and then decreased, and

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for other compounds absorption decreased with increasing salt concentration. Similar results were found by Steffen and Pawliszyn (1996) as shown in Table 2.7.

Table 2.7. Percent Change in Peak Area Due to the Addition of Salt.

Compound	15% NaCl	36% NaCl	42% NaCl
Ethanol	38	94	150
Z-3-Hexanol	220	1000	1040
Hexyl Alcohol	200	700	4200
Ethyl Butyrate	170	920	880
Linalool	300	2170	2230
Limonene	25	32	-25
α-Pinene	-37	-26	-49

Source. Steffen and Pawliszyn (1996).

Absorbent fibers such as PDMS/DVB (introduced in 1996) and PDMS/carboxen (introduced in 1997) are well suited for trace analytes (Mani, 1999). These two fiber types extract a wide range of molecular species and have high capacity. Divinylbenzene mesopores are ideal for trapping organic molecules with 6-12 carbon atoms (Mani, 1999). Carboxen is ideal for analysis of molecules with 2-12 carbon atoms, but larger molecules are strongly retained on carboxen and are difficult to desorb (Mani, 1999).

Table 2.8 Physical Properties of Divinylbenzene and Carboxen-1006.

	Surface Area	Porosity (mL/g)			
Material	(m^2/g)	macro	meso	micro	total
Divinylbenzene	750	0.58	0.85	0.11	1.54
Carboxen 1006	720	0.23	0.26	0.29	0.78

Macropore=>500 Å, Mesopore = 20-500 Å, Micropore = 2-20 Å.
Source. Mani (1999).

Sulfur compounds in water were measured by Popp et al. (1999) using 5 different fiber coatings, PDMS, PA, CW/DVB, PDMS/DVB, and PDMS/carboxen. Extraction efficiencies for the most volatile compounds were highest for PDMS/carboxen followed by PDMS/DVB. For semi-volatile compounds, PA had the highest extraction efficiency,

again followed by PDMS/DVB. The extraction efficiency for these semi-volatiles was much lower for PDMS/carboxen than for the other 4 fiber coatings. (Popp et al., 1999) also evaluated the linearity of response for the combination of SPME and GC-MS (SIM-mode) and found it to exceed 3 orders of magnitude for both PDMS/carboxen and PA in the concentration range from 20 ng/L to 20 μg/L.

Nonato et al. (2001) compared SPME with solvent extraction for the analysis of cachaca, an alcoholic drink made from sugar cane. Both methods showed highly linear relationships between concentration and detector response, but the repeatability was better with SPME (%RSD = 1.8-3.0) than with solvent extraction using dichloromethane (%RSD = 10.3-11.7). Song et al. (1998) evaluated the use of three SPME fiber coatings (PDMS, PDMS/DVB, and Carbowax/DVB) for the analysis of tomato and strawberry fruit. PDMS/DVB was the preferred coating material because it exhibited the smallest variation in partition coefficients for the volatiles and hence a more representative sample. Holt (2001) evaluated the mechanisms affecting analysis of volatiles by SPME. Selected volatiles were quantified to an accuracy of +/- 7% and the response was linear for the concentrations studied over three orders of magnitude. Jia et al. (1998) investigated the effects of sample temperature (25 to 80°C) and extraction time (5 to 40 min) on the extraction of volatiles from orange juice using a PDMS coated fiber. The time to reach equilibrium was 15 min at 80°C and increased with decreasing temperature to 50 min at 25°C. The authors also noted that as temperature increased, the total mass of volatiles extracted decreased. The authors attributed this to the fact that while the higher temperature increased the concentration of the volatiles in the headspace, it also

decreased the partition coefficient between the headspace and the fiber, so that the overall effect was to lower the mass of volatiles absorbed into the fiber coating.

Siegmund et al. (2001) monitored changes to the aroma of strawberry drink during storage using a PDMS/carboxen coated fiber. A 10 mL sample was placed in a 40 mL vial and allowed to equilibrate for 10 min at 30°C under constant stirring. The fiber was exposed for 10 min and then transferred directly to the GC injector. The split port was opened after 2 min, but the fiber was left in the injector for reconditioning for the entire 29 min run.

Sensory Analysis of Fruit Volatiles

Chemical analysis alone is not sufficient to understand the aroma differences between strawberry varieties or changes to juice or puree with heating or storage. This is because large changes in the concentration of some compounds may have little effect on aroma changes. On the other hand, changes in potent aroma compounds that may exist at levels below instrumental detection limits may have a large sensory impact.

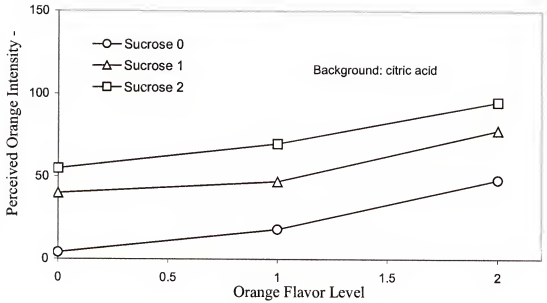
The use of human subjects for sensory analysis of flavor or aroma can be challenging. Humans have widely different sensitivities to various compounds and there may be problems with synergism or antagonism among flavor compounds. Sensory evaluation can be defined as a scientific method used to evoke, measure, analyze, and interpret responses to stimuli perceived by the human senses (Stone and Sidel, 1993).

Sensory analysis of flavor is very complex. The term flavor itself is not well defined but probably the best definition is that it is the combination of taste, aroma, mouthfeel and trigeminal responses. Taste refers to the sensations of sweet, salty, bitter and sour, impacting the taste buds in the mouth. Umami, a possible fifth taste, is often added to this list and is described as "savory." Aroma, on the other hand refers to

compounds that are sensed in the olfactory epithelium of the nose. In contrast to the 4 or 5 basic tastes, there are thousands of aroma-active compounds that give foods their distinct character. Mouthfeel describes oral sensations such as crunchiness, smoothness or other textural properties. Foods such as chili peppers, ginger, onion, or menthol stimulate nerve ends in the mouth causing perceptions such as burning, pungency, heat, or cooling (Meilgaard et al., 1999). These perceptions, known as trigeminal responses, have important modifying effects on the flavor of many foods.

Evaluation of the aroma component of strawberry flavor can be accomplished by eating the fruit or by evaluation of the aroma from the headspace above the fruit placed in a container. Evaluation of headspace alone may not provide an accurate portrayal of the aroma contribution to flavor. The sensory impact of many compounds can be changed by the process of chewing and mixing with enzymes in saliva. The increased temperature in the mouth may increase the impact of some of the less volatile compounds. For example, the aroma threshold (in water) of methional by smelling (0.2mg/kg) is much higher than the threshold of 0.04 mg/kg by tasting. In contrast, the threshold of methanethiol by smelling (0.2 mg/kg) is much lower than the threshold of 2.0 mg/kg by tasting (Mistry et al., 1997).

McBride (1990) evaluated the aroma component of orange flavored drink by varying the amount of sugar and orange flavoring. The bottom curve in Figure 2.1 shows that the perceived orange flavor of the citric acid base (no sugar) increases with the concentration of orange flavoring. However, the other two curves show that the addition of sugar had a larger effect on the perceived orange flavor than the addition of



Source. McBride(1990).

Figure 2.1 Perceived Oranginess of Mixtures of Sugar and Orange Flavoring in a Base of Citric Acid. The Three Levels of Orange Flavoring are Given on the X-Axis and Each Curve Corresponds to a Different Level of Sugar.

orange flavoring (McBride, 1990). These results have major consequences for the evaluation of the aroma component of strawberry products by tasting.

The response of the olfactory system to aromas can be described by a number of parameters. Thresholds are the limits of the sensory capacity. The absolute threshold is the lowest stimulus capable of producing a sensation. The recognition threshold is the minimum concentration of a stimulus that takes on the characteristic taste or smell of the stimulus and is often a little higher than the absolute threshold (Lawless and Heymann, 1998). Threshold values for aromas can very between individuals by as much as 2 orders of magnitude (Meilgaard et al., 1999), and the distinction between absolute and recognition threshold is probably not of great practical importance. Because of the difference in sensitivity between individuals and differences in methodology for

obtaining threshold data, published values can very considerably among authors. Most threshold values are based on the concentrations of the compounds in water, but sometimes threshold values are based on concentrations of the compound in a matrix similar to the food being studied. Ong and Acree (1998) used a retronasal aroma simulator to determine threshold values for volatile compounds in lychee (retronasal refers to the evaluation of the aroma of a compound by tasting). The device is designed to simulate the release of aroma compounds in the mouth in order to estimate the threshold that would be experienced by an individual during the consumption of a food product. Threshold values are also sometimes reported as concentrations in air. Threshold values for some important volatiles in strawberries are presented in Table 2.9.

Threshold values have been used to estimate the importance of the various volatile components in a sample by calculation of the aroma value. An aroma value, also called an odor unit, odor value, or unit flavor base, is the ratio of the concentration of an aroma compound to its threshold (Mistry et al., 1997). If the concentration of a compound is known, the aroma value can be calculated by dividing the concentration by the threshold concentration. According to this theory, the greater the aroma value, the more important the compound is to the overall aroma.

The concept of aroma value makes some incorrect assumptions based on psychophysical theories. One assumption is that two aroma-active compounds with the same aroma value will exhibit an equal perceived intensity. However, when solutions of volatiles are diluted, some intensely aromatic compounds disappear after a few dilutions steps (low aroma value), while others with a lower initial intensity may linger during further dilution giving a higher aroma value (Blank, 1997). In this case, the sensory

Table 2.9. Minimum Thresholds in Water for Selected Aroma-Active Compounds Found in Strawberries.

Compound	Threshold(ppb)	Compound	Threshold(ppb)
acetic acid	200,000 [b]	hexanoic acid	420 [a]
benzaldehyde	350-3500 [d]	Z-3-hexenal	0.25 [d]
2,3-butanedione	100 [b] 2.3-6-5 [d]	E-2-hexenal	17 [d]
butanoic acid	240 [d]	Z-2-hexen-1-ol	400 [b]
β-damascenone	.05 [b]	hexyl acetate	2 [d]
γ-decalactone	88 [c] 11 [d]	β-ionone	0.09 [a]
δ-decalactone	100 [d]	isoamyl acetate	30 [b]
γ-dodecalactone	7 [d]	linalool	25.2 [a] 6 [d]
dimethyl disulfide	0.16-12 [d]	mesifuran	
dimethyl trisulfide	.005-.01 [d]	methional	0.2 [d]
ethyl-isobutyrate	15 [a]	methyl butyrate	60-76 [d]
ethyl butyrate	20 [a]	methyl caprylate	200 [d]
ethyl cinnamate	1.1 [a]	me-2-methylbutyrate	0.25 [d]
ethyl hexanoate	14 [a] 1.0	methyl hexanoate	70-84 [d]
ethyl propanoate	10 [d]	nonanal	1 [d]
ethyl 2-methylbutanoate	18 [a] 0.1-0.3 [d]	nona-2E,4E-dienal	0.09 [d]
ethyl 2-methylpropanoate	0.1 [d]	E-2-nonenal	0.08-0.1 [d]
eugenol	6-30 [d]	nonanoic acid	3000 [d]
DHF	0.04 [d]	1-octen-3-ol	1 [d]
geraniol	30 [b]	octanal	0.7 [d]
heptan-2-one	140-3000 [d]	octanoic acid	500 [a]
hexanal	4.5-5 [d]		

[a]Ferreira et al. (1998), [b]Guth (1997), [c]Etievant et al. (1999), [d]Leffingwell & Assoc. (2002)

impact of the later compound will be overestimated. Another assumption is that there is a linear relationship between a compound and its perceived intensity. This assumption is contrary to both Fechner's Law and Stevens' Law, which states that a sensory magnitude increases with stimulus intensity raised to a power. For aromas:

$$\text{Aroma Intensity} = k\,(C-T)^n$$

Where k is the constant of proportionality, T is the compound's threshold, C is the concentration of the compound.

The concept of aroma value suggests that k and n in the above equation are both 1 for all compounds and that the aroma intensity varies directly with an increase in

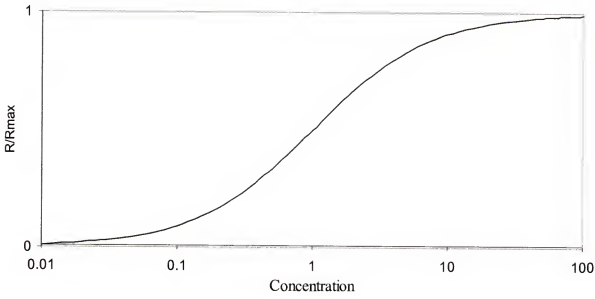
concentration. However, studies such as those by da Silva et al. (1994) have shown considerable differences between compounds with regard to changes in perceived aroma intensity with changes in concentration. Berglund et al. (1971) reported values of n in the above equation of between 0.13 and 0.72 for 28 aroma-active compounds. This contrasts to the aroma value concept, which would suggest a uniform value for n of 1 for all compounds.

Equations such as Stevens' Law fit observed data fairly well, but the relationship is still only empirical. Another dose/response relationship based on physiological theory has been proposed by McBride (1987). He suggested that the taste response might involve the binding of a molecule to a receptor in a manner similar to enzyme substrate binding following Michalis-Menton kinetics. The relationship first proposed by Beidler, a pioneering physiologist is given by:

$$R = (Rmax * C)/(k + C)$$

Where R = response; Rmax = the maximal response; k = the concentration at which the response is half-maximal; and C = molar concentration.

In a plot of log concentration (Figure 2.2), the relationship forms an s-shaped curve. In this relationship, response is fairly flat in the area near the detection threshold, followed by a steep rise and finally a leveling off at an upper limit. The relationship which has an upper limit representing a saturation of the taste response would seem to be a better model than the empirical Stevens' Law, which predicts the response would continue upward indefinitely (Lawless and Heymann, 1998). The upper limit is also called the terminal threshold and is the magnitude of a stimulus above which there is no increase in perceived intensity (Meilgaard et al., 1999).



Source. Meilgaard et al. (1999).

Figure 2.2. The Sigmoidal Relationship Between Taste Response, R/Rmax, and Stimulus Concentration, C, as Specified by the Beidler Equation; k is Set Equal to 1 for Convenience.

There is another important threshold called the difference threshold. It is the extent of change in the stimulus necessary to produce a noticeable difference (Meilgaard et al., 1999). This threshold is an important concept for designing and evaluating sensory methods such as discrimination tests or descriptive analysis.

Discrimination Techniques

The purpose of a discrimination test is to determine if people can detect a difference between samples. Discrimination tests are often used to determine if there is a change in the sensory properties of a product due to processing or storage. These tests are most useful when the differences between samples are subtle. Three commonly used discrimination tests are the duo-trio test, the triangle test and the difference from control test. A reference and two coded samples are presented in the duo-trio test. The panelist is asked which of the two coded samples is different from the reference. Siegmund et al.

(2001) used duo-trio tests to evaluate strawberry drink during storage. After 3 weeks of storage at 37°C (corresponding to a storage time of 6 months at room temperature) significant sensory differences were observed. The changes were described as a loss of fresh fruity and strawberry like aromas and an increase in stale and musty attributes. Paakkonen and Mattila (1991) used duo-trio tests to evaluate the quality of freeze-dried strawberries. The panelists were asked to rate the aroma and flavor intensity of the sample against a reference and comment on sourness, off-aromas and off-flavors. Panelists were asked if the sample was stronger, equally strong, or weaker and if it was less pleasant, equally pleasant or more pleasant than the reference. The duo-trio test was used by Pyysalo et al. (1977) to determine aroma thresholds for some compounds important to strawberry.

In the triangle test, three coded samples are presented and the panelist is asked to choose which sample is different. The chance of guessing correctly in the triangle test is 1 out of 3 compared to 1 out of 2 for the duo-trio test. Therefore the triangle test is statistically more efficient than the duo-trio test (Meilgaard et al., 1999).

Another important discrimination test is the difference-from-control test. In this test, one sample is designated the "control" and all other samples are evaluated with respect to how different each is from the control. This test can be used for more than two samples and has the additional advantage that the degree of difference can be quantified.

Descriptive Analysis

Descriptive analyses are the most complex sensory tests used to evaluate food products. They are often used to compare similar competing products, to evaluate new products during the development phase, to monitor product quality during shelf-life studies, or to help determine the cause of customer complaints (Stone and Sidel, 1993).

Descriptive analysis can focus on specific areas such as textural differences, flavor differences, or differences in appearance or they can be used to give a complete overall description of a product. The objective of descriptive analysis is "to find a minimum of descriptors that will convey a maximum amount of information regarding the sensory characteristics of the product" (Stampanoni, 1993, page 19).

The Flavor Profile method, developed in the 1940's by Arthur D. Little, Inc., was one of the earliest methods. Flavor Profiling is a consensus technique using a panel leader and four to six judges. The judges are first screened for their sensory acuity and then trained using a variety of products from the product category being evaluated. Following training, the group meets for about an hour, discusses the products, and produces a consensus report in graphical or tabular form that is not designed to be quantitative for the various descriptors.

The developers of the method suggest that the combined professional judgment of the panel would be adequate and that statistical analysis would be unnecessary (Stone and Sidel, 1993). Although this method is often criticized for lack of statistical treatment, it can be useful for quickly screening large numbers of products, especially if the sensory panel is very familiar with the product category being tested.

Quantitative Descriptive Analysis (Stone and Sidel, 1974) and Sensory Spectrum (Meilgaard et al., 1999) are two more modern statistical techniques that are most often cited in the literature, but many other methods such as Quantitative Flavor Profiling (Stampanoni, 1993) have been developed. No one method dominates and many modifications or hybrids of these techniques are used. Differences in descriptive methodology arise due to differences in the products being analyzed, the experience of

available panelists, the advantages and disadvantage of different measurement techniques, the available time and money, and the goals or biases of the researchers. However, all these techniques follow the same basic methodology; selection of panelists, determination of the attributes for analysis, training of panelists, measuring the reliability and reproducibility of the panelists, and product testing and analysis.

Because of the time and expense involved in training, panelists are first selected based on their long-term availability and their interest in the product category. It is important that the panelists like the product or are potential consumers of the product. If they are not familiar or interested in the product, panelists tend to be less sensitive and more variable in regard to product differences (Stone and Sidel, 1993). Next, the panelists need to be screened based on their ability to mentally isolate and quantify specific sensory attributes from a complex matrix and their ability to accurately communicate their perceptions. With Quantitative Descriptive Analysis (QDA), panelists are screened using discrimination tests. Typically, potential panelists are presented with 20 to 30 triangle tests to evaluate their ability to determine differences between similar samples. In addition to discrimination tests, panelists for the Spectrum method are selected using written tests to measure the person's ability to accurately assign scalar ratings and ability to describe the characteristics of sample products.

The panelists that make it through the screening process are used to determine the attributes that are important in describing the product. Selection of attributes usually consists of exposing the panelists to a wide range of products within the category. The panel leader in QDA asks the panelists to privately develop their own list of terms to describe the products and then the panel meets to discuss these terms. Typically 10 to 12

evaluators sample a wide range of products one at a time in separate booths and panelists do not interact. The evaluators then get together and review all the suggested terms and decide on a consensus group of attributes to use for the testing. The panelists then decide on definitions of the terms and agree on how the attributes are to be quantified between the anchors. The panel leader does not play a roll in the development of the descriptive terms because it might unduly influence the panel. The purpose of this method of developing terms is to produce a descriptive language that is close to consumer language. This facilitates communication of the results of the trials to interested individuals that may not have technical sensory backgrounds. However, this method can result in ambiguous terms such as "creamy" which might be applied to smoothness, viscosity, fatty mouthfeel or cream flavor (Lawless and Heymann, 1998). Panelists may also be frustrated if their terms are not used in the testing phase.

An alternative to QDA, is to train the panelists to use more specific "scientific" terms. The panelists in the Spectrum technique pick attribute names from an existing list of previously developed descriptors. These terms have a written definition and are usually associated with a physical standard. The use of previously developed terms shortens the training time and the descriptive terms can be related to product ingredients, thereby simplifying changes in product formulation. Lists of descriptors for some products such as wine have been developed. However, such pre-existing descriptors are not available for all products. If this is the case, the Spectrum method has the panel develop new terms in a manner similar to QDA.

Another method, Quantitative Flavor Profiling (QFP) avoids the use of panelists to develop new terms by using a group of experienced panelists to determine the

descriptors for a specific product in a manner similar to classic Flavor Profiling. The use of a separate group to define the attributes has the advantage of focusing the panelists on the objectives of the research. It reduces the training time and has the advantage that the panelists do not have any preconceived ideas or biases since they are not involved in the development of the descriptors (Stampanoni, 1993).

Following the selection of attributes, the judges are trained to quantify the levels of these attributes in the products being tested. In QDA, the panelists are trained to use a line scale. The line scale (15 cm) is anchored at each end with word descriptors that indicate increasing intensity of the attribute from left to right. For example "weak" could be used as the left anchor and "strong" as the right anchor. Panelists make a mark on the line indicating the intensity of the attribute. Panelists are not highly trained to provide absolute magnitudes for each attribute. In QDA, it is assumed that evaluators will use different parts of the scale and therefore relative differences and consistency are emphasized rather than absolute scale values. The line scale was chosen to avoid number bias and because responses are thought to be normally distributed and more amenable to parametric statistical analysis.

Quantitative Flavor Profiling also uses a line scale, but the scales are anchored with a standard representing a specific intensity generally at about 75% of the scale (Stampanoni, 1993). An example of standards and descriptors used for QFP of strawberry flavored yogurt is shown in Table 2.10. In contrast to the other methods, QFP makes this standard available to panelists both during training and during testing. This is thought to reduce panelist day-to-day variability and variability between panelists (Stampanoni, 1993). Methods similar to QFP are commonly used for descriptive analysis

of wines. References for wines have become fairly standardized and are commonly used for wines such as Pinot Noir (Guinard and Cliff, 1987).

The Spectrum method trains panelists to score their perceived intensities with reference to pre-learned "absolute" intensity scales (Meilgaard et al., 1999). It is difficult

Table 2.10. Standardized Flavor Language for the Quantitative Description of Strawberry Flavors Using the QPF Technique: Terminology and References.

Fruity-Green	Trans-2-hexenal (0.5%TA)
Grass-Green	Cis-3-hexenol (1%PG)
Ripe-Fruity	Ethyl butyrate (1%PG)
Jammy-Cooked	Strawberry juice concentrate (Pure), Strawberry jam
Floral	Linalool (1%PG)+ Hedione (0.1%PG)) 1:1
Estery-Candy	Amyl-iso-acetate (1%PG)
Raisin	Davana oil (1%TA)
Sweet-Cotton Candy	Ethyl maltol (Pure)
Vanilla	Ethyl vanillin (Pure)
Buttery	Diacetyl (1%PG)
Creamy	UHT Cream- 35% Fat
Musty	Cola –infusion in ethanol (Pure)
Methyl Anthranilate	Methyl anthranilate (1%PG)
Juicy	Acetaldehyde (10 ppm H2O) (by mouth)
Hay-Like	Black Tea (Lipton quality no. 1), Hay-oil (10%VO)
Lactony	Gamma-decalactone (1%PG)

TA = Triacetin, PG = Propylene glycol, VO = Vegetable Oil.
Source. Stampanoni (1997).

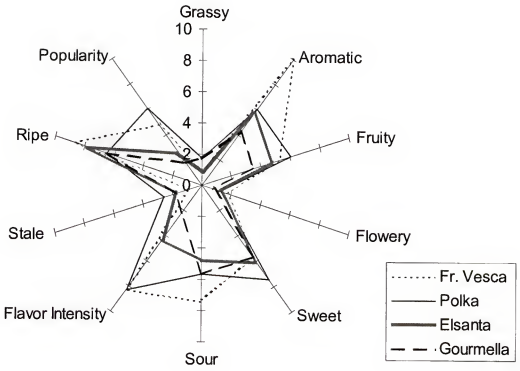
to train panelist to use absolute scales and training requires 3 to 4 hours per week for 14 weeks. Typically, from 2 to 5 specific reference points are used to anchor a 15-point numeric scale for each attribute. Using reference points to calibrate the panelists would be akin to using pH standards to calibrate a pH meter (Lawless and Heymann, 1998). Theoretically, the Spectrum method should make results between different sensory panels as comparable as pH measurements in different laboratories. However, while human subjects are good at relative judgments, they are poor at absolute judgements, and the goal of absolute measurements may not be possible in practice.

The next step in training is to have panelists practice rating the samples using the same ballot and methodology that is used during the actual testing phase. In addition to giving the panelists practice, most descriptive methods use this step to evaluate the consistency of the panelists. The data from these sessions are analyzed for the significance levels of the interaction among panelists. Additional training is given to panelists that give significantly different results from the majority of the group.

After the training is complete, and the panelists are producing consistent results, evaluation of the samples can begin. The samples are given random codes and are presented using standard sensory practices such as proper lighting, use of private booths, and the use of water and a bite of cracker to cleanse the palate between samples.

Analysis of variance (AOV) is usually used to evaluate the data, but multivariate statistical techniques such as principal component analysis (PCA), partial least squares (PLS) or multivariate analysis of variance (MANOVA) are commonly used. Data can be presented in tabular form or in a graphical form such as a radar plot (Figure 2.3).

The complexity of descriptive analysis should allow for the comparison of the sensory attributes of a product with instrumental analysis. However, this concept is based on the assumption that the attributes used are independent of each other and that the panelists can separate them from the complex mixture (Murray et al., 2001). However, identifying components in mixtures is difficult for most people. The ability of humans to identify components in complex mixtures was evaluated by Laing and Francis (1989). They found that participants correctly identified all 3 aromas in a 3 component



Source. Ulrich et al. (1997).

Figure 2.3. Radar Plot Comparing Descriptive Profile of Four Strawberry Varieties.

mixture only 14% of the time. The ability to identify all 5 compounds in a 5 component mixture fell to essentially zero. Livermore and Laing (1996) evaluated this same ability in professional perfumers and flavorists and concluded that the ability to identify all the components in a mixture of more than three components could not be improved even after years of training and experience. However, the inability to identify individual aromas in mixtures does not mean that panelists cannot tell the difference between samples of complex mixtures. Jinks and Laing (1999) evaluated the ability of subjects to determine if a specific component was present or absent in mixtures containing up to 16 aroma active compounds. The concentrations were adjusted for each participant so all compounds in the mixtures were present in similar perceived intensities. Participants had some ability to determine the presence or absence of a compound in mixtures of up to 12

components, but not in mixtures of 16 components. Even though each compound in the mixture was adjusted to have equal intensity, some compounds were easier to pick out than others. The fact that some compounds dominate in mixtures has been reported by other authors (Kendall and Neilson, 1966; Czerny et al., 1999).

In spite of these difficulties, descriptive analysis provides a relatively complete evaluation of the important parameters contributing to the difference between samples.

Integrating Chemical and Sensory Analysis

Three of the most common statistical methods for relating sensory data to instrumental data are multiple linear regression (MLR), principal component analysis (PCA), and partial least squares regression (PLS). Boccorh et al. (1999) developed a model of blackcurrant flavor intensity using PLS. A set of 37 volatile components previously identified as aroma-active were quantified in 133 blackcurrant concentrates which were also analyzed by a sensory panel. The final model had a regression coefficient of 0.8 and was dependent on 10 flavor components. da Silva and das Neves (1997) used PCA to evaluate the differences in volatile components of strawberries. The authors were able to distinguish between varieties based on their relative content of methyl-2-methyl butyrate, ethyl-2-methyl butyrate, methyl butyrate, ethyl butyrate and methyl hexanoate. Togari et al. (1995) compared the sensory properties of tea to gas chromatographic data using MLR, PLS, and principal component regression (PCR). Models for each of the sensory descriptors were evaluated using each the multivariate calibration methods. The accuracy of PLS in estimating regression coefficients was better than the accuracy of PCR and nearly equivalent to those obtained by MLR. Models with high regression coefficients were developed for most of the sensory

characteristic except for the term resinous. The authors attributed this to the panelists inability to accurately score resinous, due to its ambiguity.

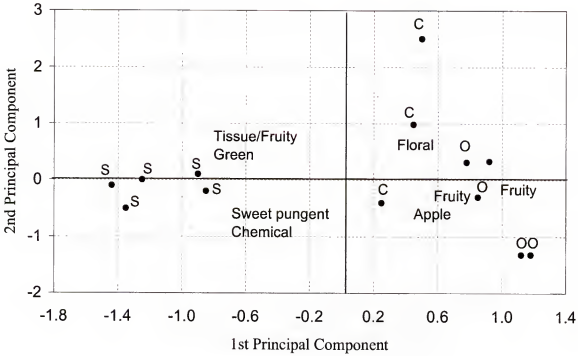
Principal component analysis is typically used to simplify a data set. When PCA is used, the original variables are converted into a new set of "components" which are linear combinations of the original variables (Chien and Peppard, 1993). These principal components have no real physical meaning. Typically, the samples and the variables are plotted on graphs using the first 2 principal components as the x-axis and y-axis. The score plot (for samples) reveals how the samples are related to each other and the loading plot (for variables) shows the contribution from each of the original variables to the principal components (Chien and Peppard, 1993). Because PCA reduces the number of variables to a few principal components, it reduces the complexity of the data set, making it much easier to see how samples are correlated with each other.

It is important to note that a correlation does not imply cause and effect. For example Krumbein and Auerswand (2000) found a very high correlation ($R = 0.73$) between the sensory descriptor "fruity" and the compound 1-octen-3-one. The correlation does not indicate if this compound (which has a strong mushroom aroma) contributes to the fruity aroma or if its concentration simply changes in the same way as other compounds that actually produce the fruity aroma.

Gas Chromatography/Olfactometry

Gas chromatography offers researchers a method of separating individual volatile components from a mixture and is the first step in identifying the many compounds that make up the aroma of foods. The development of capillary columns in the 1970's allowed for more efficient separation and subsequently thousands of volatile compounds were identified (Mistry et al., 1997). It was soon realized that foods were much more

complicated than first thought and simply cataloging compounds did little to provide an understanding of the complexity of aroma (Acree et al., 1984). One of the most obvious problems was that the identification of a compound does not by itself indicate whether



Source. (da Silva and das Neves, 1999).

Figure 2.4. Graph of Principle Components for Samples Strawberry cv. Selva (S), Oso Grande (O), and Chandler (C) as a Function of 12 Compounds Determined to be Important to Strawberry Flavor, Projected in the Planes Defined by the First and Second Principal Components. Variable Loading is Labeled According to the Corresponding Sensory Descriptions.

the compound even has an aroma. In fact, the only way to tell if a compound has an aroma is for someone to smell it. Even before the introduction of capillary columns, researchers were smelling the effluent from packed columns in an attempt to identify the "character impact" compounds of various commodities. However, these character impact

compounds proved to be rather elusive and it became necessary to develop formalized systems to evaluate the contribution of the many aromas in food (Mistry et al., 1997).

Gas chromatography/olfactometry (GC/O) describes a system that uses a gas chromatograph to separate volatile compounds from a complex matrix such as foods, and then uses a human nose as a detector (Blank, 1997). The human nose is extremely sensitive to many aroma-active compounds. For example, 2 methoxy-3-hexyl pyrazine has an aroma threshold of 1 part per trillion in water (Reineccius, 1994). Meilgaard et al. (1999) estimates that there may be thousands of aroma-active substances for which the nose is 10 to 100 times more sensitive than the most sensitive gas chromatographic method. In a GC/O study of wine, Miranda-Lopez et al. (1992) found that over 50% of the aroma-active peaks were not detected by FID, and only 20% of the FID peaks were aroma-active.

In GC/O systems, the flow from the column can go directly to a sniff port or the flow can be split to go simultaneously to the sniff port and another detector. Sniff ports are designed to mix the flow from the column with warm humid air and route the flow to the human subject so that he/she can sit comfortably while concentrating on sniffing aromas as they elute from the column. van Ruth and O'Connor (2001) evaluated the influence of factors such as chromatography conditions, sniff port flow, and training on GC/O results.

GC/O has been formalized into two main techniques, dilution analysis and time-intensity methods. Another methodology, detection frequency, also has merit. There are two main types of dilution analysis, aroma extract and dilution analysis (AEDA) and Charm Analysis. Both techniques rely on repeatedly diluting the sample and performing

GC/O analysis until all the aromas disappear. The number of dilutions required for an aroma to disappear is related to the importance of the aroma. The sample is stepwise diluted, typically by a factor of 3 until the final dilution results in the lack of any detected aroma. For example, if the sample is continuously diluted 3:1 five times, the dilution value becomes $3^5 = 243$. It is important to note that dilution analysis is only a rough estimate and values can easily vary by at least one dilution step (Blank, 1997). In the example above, with a 3:1 dilution, the estimate would only be within about 300%.

In AEDA, the dilution value is an estimate of the number of times the concentration of the compound exceeds its detection threshold and is termed the flavor dilution value (FD). A FD chromatogram is then plotted using an indexed retention time on the x-axis versus the FD factor (or the log of the FD factor).

In Charm Analysis, the evaluator responds to an aroma-active compound by depressing a button and holding it down until the aroma disappears (Acree et al., 1984). The evaluator then either selects an aroma descriptor from a list on the computer screen or verbalizes the descriptor to an assistant or a recording device. At each retention time where a sensory response is repeatedly recorded (with the associated descriptor), the length of time the aroma is present for each dilution is then summed to produce a value similar to a peak area on a GC chromatogram. The reporting of peak area rather than peak height is used to adjust for the fact that some compounds chromatograph poorly and have low broad peaks and their sensory impact would be underestimated if the response was recorded as a peak height (Mistry et al., 1997).

The main difference between Charm and AEDA is that Charm takes the duration of perception into account whereas AEDA does not (van Ruth, 2001). This can result in

slightly different results when the two methods are used on the same sample. Abbott et al. (1993) compared Charm and AEDA for the analysis of beer samples. They found that the order of importance of the key odorants was different for most of the panelists when results were presented as FD factors compared with Charm values. Also, since it proved difficult for panelists to determine the end of an aroma-active region, the Charm values were subject to greater error. Dilution methods essentially provide an estimate of the number of times the concentration of the compound exceeds its threshold concentration. It is assumed that the higher the FD factor or Charm value for a compound, the more important the compound is to the overall aroma (van Ruth, 2001). However, since FD factors and Charm values are analogous to the aroma values previously discussed, they are subject to the same criticisms.

Instead of relying on serial dilutions, time-intensity methods are based on magnitude estimation of the aroma intensity. One such method, Osme, has the evaluator rate the strength of the aroma using an electronic time-intensity scaling device (Miranda-Lopez et al., 1992). The device has a 15 cm slide scale and intensity labels from 0 = none to 15 = extreme. The evaluator's response is recorded directly into a computer interface that produces a graph of time/intensity measurements just like an electronic detector. As originally developed, Osme uses 4 trained observers performing 4 replications each. Peaks detected at least 50% of the time by a given panelist and by at least 3 of the 4 panelists are then put together to form a consensus Osmegram. Da Silva et al. (1994) evaluated the quantitative capability of Osme using model solutions of six aroma-active compounds. All four evaluators were able to establish a statistically significant relationship between aroma intensity and concentration for five of the six compounds.

The relationship was well fit by linear, logarithmic, and power functions, and it was not clear which was the best fitting function. Another intensity method uses cross-modality matching with finger span (Etievant et al., 1999). An electronic device that measures the distance between the thumb and fore-finger is used and panelists are asked to match the aroma intensity by moving the fingers farther apart for stronger intensities. Evaluation of model solutions showed large variations between evaluators and between sessions for each evaluator, but the perceived intensity/concentration ratio showed good agreement with Stevens' Law. In the finger span method, evaluators are asked only to estimate the magnitude of the aroma whereas in Osme, the evaluators are asked to record the aroma magnitude throughout the time the aroma is present. Other simpler methods of magnitude estimation are also used. Lesschaeve et al. (1991) used five evaluators to simply rate the intensity of aromas eluting from the chromatograph on a scale from 1 to 4, without the use of electronic recording devices.

Methods based on the frequency of detection of an aroma have also been described. A recently developed method referred to as olfactometry global analysis (Le Guen et al., 2000) uses a large number of untrained observers. Unlike the other methods, the importance of an aroma is linked to the frequency of detection among the panelists. Theoretical justification of the method is presented by Pollien et al. (1997). Studies have shown that frequency of detection techniques compare favorably with both dilution methods and intensity methods. Van Ruth and O'Connor (2001) compared detection frequency with perceived intensities for eight evaluators. Detection frequency was highly correlated ($r = 0.971$) with aroma intensity ratings. Le Guen et al. (2000) compared detection frequency with Osme and AEDA. Their results showed that the methods were

positively correlated with p values ≅ 0.00001. The authors also commented that Osme was the most accurate method and the detection frequency method was the easiest and most rapid.

All of these methods have their advantages and disadvantages. Dilution methods tend to rely heavily on a single evaluator because repeated analysis is very time consuming (van Ruth, 2001). It has been shown that humans differ in their sensitivity to many compounds, and an exhaustive analysis of one evaluator's sensitivity may not be as valuable as a less intense evaluation by a number of different people. Also, the time required for repeated dilution takes away time that could be spent running the sample under different chromatographic conditions such as using different phase columns. Performing GC/O analysis on two different columns provide important additional information. Compounds that may elute with the solvent on one column may be detected on another column. Compounds that may elute together on a DB-5 column such as E-2 hexenal and ethy-2-methyl butyrate may be separated on a carbowax column. Some compounds also chromatograph poorly on some columns, producing faint wide peaks that are hard to detect, while the compound may produce high narrow peaks on another stationary phase. Problems such as cross-adaptation, where one compound affects the aroma of a similar compound eluting soon after, can be detected by performing GC/O on different phase columns. However, dilution analysis has an advantage over time/intensity methods because the evaluator is asked only to determine if an aroma is present and to describe the aroma. Therefore, dilution techniques are free from the complexities inherent to methods requiring estimates of stimulus intensity (Acree et al., 1984). Estimating intensity can be a difficult task when aromas elute in quick succession, each

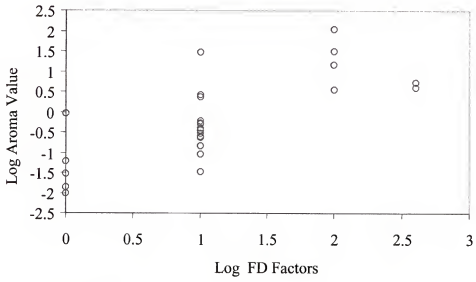
one only lasting for a few seconds (Acree et al., 1984). In spite of the differences in these methods, a comparison of detection frequency, Osme, and AEDA showed that the three methods were very similar and well correlated (Le Guen et al., 2000). The use of GC/O is subject to a number of limitations. The olfactory data are only as good as the sample that is analyzed. Since all extraction methods are somewhat selective, the sample cannot be identical to the food product. Often, highly volatile compounds are not evaluated because they are lost during the concentration of the extract or are masked in GC/O by the solvent peak. Static headspace extraction is probably the closest representation of the aroma of a food product, but it is limited since only very dilute samples are obtained. Grosch (2001) recommends that AEDA analysis be combined with static headspace analysis to produce a more complete picture of the volatile profile. The importance of using both a concentrated extract and static headspace analysis is illustrated by a study of stewed beef juice (Guth and Grosch, 1994). In this study, the three most important aroma compounds determined by AEDA were DHF, butyric acid and acetic acid, but neither acetic acid nor butyric acid were listed as important in the static headspace analysis. The most important compounds determined by static headspace analysis were acetaldehyde and methanethiol but neither of these compounds was listed as important in AEDA. This study certainly shows the importance of having a representative sample.

GC/O of aroma extracts may still produce errors in the estimation of the importance of various volatiles to the overall profile, even with a very representative extract. This is because the aroma-active compounds are completely volatized in the hot injector and the aroma impact of a compound can be magnified significantly compared to the impact of the same compound dissolved in a food matrix at room temperature.

Ferreira et al. (1998) compared FD factors from AEDA to estimated aroma values. An aroma value is calculated by dividing the concentration of the compound in the headspace by its measured threshold concentration. The concentration in the headspace depends on how the compound partitions between the headspace and the sample matrix. A FD factor is an estimate of the aroma value based on the amount of dilution of an extract necessary for the aroma of a compound to no longer be detected (to go below its threshold). The FD factor is calculated from a fully volatized sample and the effect of the partition of a compound between the gas and liquid phase is not taken into account. Because GC/O does not take into account extraction efficiency and the fact the compounds are fully volatilized in the GC injector, FD factors may poorly represent the aroma-impact of some compounds. Figure 2.2 shows how poorly FD factors can correlate with aroma values which should be better estimates of the aroma activity of a compound in a food matrix. Ferreira et al. (1998) developed a method based on the retention times of volatiles on both polar and non-polar phase gas chromatography columns. The equations developed could be used the improve the estimation of aroma values from GC/O data.

GC/O has also been criticized because the importance of a compounds is based on its individual aroma, which might be different from the impact of the compound in a food matrix. For example, in a study of coffee aroma, Czerny et al. (1999) found that a cooked potato aroma, that they attributed to methional, was masked by the furanone compounds. The authors also found that 4-vinylguaiacol was important to the aroma only because it masked a strong sulfurous note.

Fatigue could be problem in GC/O analyses, especially during long runs. However, van Ruth and O'Connor (2001) investigated the effect of fatigue by



Source. Ferreira et al. (1998).

Figure 2.5. Comparison of Flavor Dilution Factors Based on AEDA Analysis of Freon 11 Extracts of Grenache Red Wine to Aroma Values Estimated from Thresholds and Measured Headspace Concentrations Above the Sample.

comparing results from 45 minute runs to results where the evaluators only evaluated the last half of the run. Their results found no significant differences between results from the full and half runs and concluded fatigue was not a problem in the longer runs.

One important part of GC/O is the reporting of retention indicies of compounds based on a series of alkane standards rather than retention times (Van Den Dool, 1963). This step is important because retention times of aromas must match up throughout the dilution series and it also links the sensory response to a reproducible chemical property.

Since many important aroma-active compounds and their associated retention times (Kovats index) are reported in the literature, calculating the index of an unknown

Table 2.11 Retention Indicies for Compounds Reported in Strawberries.

Compound	Kovats DB-5	Kovats Wax	Kovats FFAP
acetic acid			1435 [b] 1451 [d]
2,3-butanedione	600 [b]		967 [b] 987 [d]
butanoic acid(butyric)	820 [d]	1630 [e]	1613 [b] 1620 [f]
β-damascenone	1395 [b]	1832 [c] 1790 [e]	1795 [b] 1805 [d]
γ-decalactone	1472 [d]	2109 [e]	2140 [d]
δ-decalactone	1493 [d]	2217 [c]	2194 [d] 2125 [f]
ethyl butyrate	802 [a]	1040 [a]	1023 [b] 1031 [f]
ethyl cinnamate(trans)	1460 [b]	2149 [c]	2125 [b]
ethyl hexanoate	1000 [a]	1240 [a]	1225 [b]
ethyl 2-methylbutanoate	850 [a]	1055 [a] 1041 [e]	
ethyl 2-meth-propanoate	756 [a]	969 [a]	
eugenol	1355 [b]	2186 [c]	2159 [b]
DHF	1060 [a]	2043 [a]	2023 [f]
hexanal	800 [g]		1071 [e] 1079 [f]
hexanoic acid	1020 [c]		1835 [b] 1830 [f]
Z-3-hexenal	793 [d]		1147 [d]
E-2 hexenal	853 [d]	1207 [e]	1215 [d]
Z-3 hexen-1-ol	855 [a]	1398 [a]	
E-3 hexen-1-ol	851 [g]		
hexyl acetate	1008 [g]		1268 [f]
β-ionone	1485 [g]		
linalool	1100 [a]	1565 [c] 1548 [e]	1535 [b] 1543 [f]
mesifuran	1057 [a]	1600 [a]	
methional	905 [a] 977 [c]	1456 [a] 1469 [c]	
methyl anthranilate	1343 [c]	2255 [c]	
methyl butyrate	724 [g]		
2-methylbutanoic acid	856 [a] 874 [d]	1681 [a]	1657 [b]
methyl hexanoate			1183 [f]
β-myrcene	991 [a]	1167 [a]	
E-nerolidol,	1564 [g]		2034 [f]
nonanal		1380 [e]	1386 [f]
E-2-nonenal	1158 [c]	1545 [c] 1519 [e]	1527 [b]
1-octen-3-one	978 [a]	1307 [a]	1288 [d]
octanal			1272 [e]
octanoic acid	1200 [c]	2083 [c]	2051 [f]

[a]Siegmund et al. (2001), [b]Guth (1997), [c]Aznar et al. (2001) [d]Derail et al. (1999), [e]Ong and Acree (1998), [f]Lambert et al. (1999), [g]Adams (1989).

compound is often a first step in identification. Retention indicies for many of the compounds reported in strawberries have been collected in Table 2.11. Using GC/O

and comparing retention index values from chromatographic runs on two different columns it is possible to identify trace compounds even when there is no FID peak.

A summary of GC/O analysis of strawberries (both fresh and processed) was presented earlier in Table 2.1. The differences in reported compounds could be due to cultivar differences, the treatment of the fruit before extraction, or the volatile extraction methods used. Ethyl butyrate, methyl butyrate, ethyl hexanoate, E-2-hexen-1-ol, butanoic acid, 2-methyl butanoic acid, DHF, mesifuran, and linalool were the only compounds reported by 3 or more of the 5 authors.

Aroma Recombination

GC/O provides valuable information on the important aroma-active volatiles in a particular food; however, it does not provide a very accurate picture of the true aroma profile. The next step in understanding a food aroma is to identify and quantify the aroma-active compounds. This is often a difficult task since many important volatiles are present at levels below the detection limit of common analytical instruments. For volatiles at trace levels, stable isotope dilution assay is the most accurate method of quantification (Mistry et al., 1997). In this method, a known quantity of a labeled isotope of the aroma-active compound is added to the sample and the volatile fraction is isolated. The analyte and its labeled counterpart are then analyzed by selected ion mass spectrometry (SIM) in the chemical ionization mode. Assuming that the analyte and its isotope undergo the same losses during isolation, the SIM peak areas can be compared and their relative concentrations calculated. Chemical ionization and SIM provide a more sensitive analysis then normal electron ionization mass spectrometry. Schieberle and Hoffman (1997) used stable isotope dilution assay to quantify volatiles in fresh strawberry samples.

GC/O dilution analysis can also be used to estimate concentrations of trace compounds. Mistry et al. (1997) used GC/O to estimate the concentration of geosmin in extracts of mold infected grain. By this method, a standard of geosmin is prepared at a known concentration that can easily be detected by a number of evaluators. Each evaluator then randomly sniffs diluted samples of the standard, and the dilution at which there is no longer a perceptible aroma is the threshold for that evaluator. Next, the unknown sample and dilutions are sniffed until there is no perceptible aroma in a sample dilution. The geosmin concentration in the unknown sample is then estimated by multiplying the dilution factor at which there is no longer a perceptible by the threshold value determined using the standard concentrations. The volatiles that can easily be detected by analytical instruments can be estimated by using common methods such as the use of internal or external standards or by the method of standard addition.

Aroma-recombination studies are performed by combining key aroma compounds in a matrix similar to the natural food and then sensory studies are performed on the mixed samples. It is important that compounds are combined in a matrix similar to the food product because the volatility and hence the aroma of compounds is dependent on the matrix. For example, de Roos and Nelissen (2000) found the static headspace concentration of some compounds was up to 800 times higher over water than over cream. Tandon et al. (2000) also found very different aroma thresholds for tomato volatiles when the compounds were dissolved in water compared to the compounds dissolved in tomato homogenate.

In spite of the importance of confirming GC/O data with aroma recombination studies, only a few experiments are reported in the literature. The simplest experiment is

to simply compare the test mixture to the actual food product. Buttery et al. (1990) used GC/O methods to determine the most important aromas and their concentration in tomato puree. The authors made a "synthetic paste essence" consisting of the seven most important compounds dissolved in water and evaluated the aroma using a sensory panel. The panel found that the model solution closely duplicated the aroma of tomato paste. Recombination studies based on GC/O are not always totally successful. Bazemore (1998) made model solutions of the most important aroma-active components of orange juice as determined by AEDA. The models were based on the concentrations of these compounds reported in the literature, but the model solution did not resemble the flavor of orange juice. Upon further experimentation, the author found that acetaldehyde, ethanol, limonene, α-sinensal and β-sinesal were required to simulate orange juice aroma even though only the sinensals were detected by GC/O (but their intensities were not strong).

A more involved type of aroma recombination study compares samples with a single missing compound to a complete sample. These experiments can reveal compounds that were thought to be important (having high aroma values) but were suppressed or masked in the overall aroma model (Grosch, 2001). Studies on butter (Schieberle et al., 1993), Swiss cheese (Preininger et al., 1996), French fries (Wagner and Grosch, 1998), and stewed beef juice (Guth and Grosch, 1994) have been reported. Aroma recombination studies can produce unexpected results compared to the aroma importance determined by GC/O. Based on AEDA, Wagner and Grosch (1998) expected methional (which has a strong cooked potato aroma) to be important to the aroma of French fries. However, when this compound was omitted from the model mixture,

panelists could not tell a difference from the complete model mixture. Even more interesting, is the fact that when methanethiol (which has a cabbage-like aroma) was left out of the model, all the panelists found the aroma impression of boiled potatoes lacking. Guth and Grosch (1994) evaluated the aroma of stewed beef juice using AEDA and static headspace analysis. The data from the two different GC/O methods produced very different results. Aroma omission model studies showed that neither GC/O method

Table 2.12. Sensory Descriptors, Aroma Thresholds, and Concentration of Major Aroma Impact Compounds in a Model Strawberry Juice Mixture.

Compound	Descriptor	Threshold (mg/L)	Conc. (mg/L)
DHF	caramel-like	10	8740
Z-3-hexenal	green, leaflike	0.25	330
methyl butanoate	fruity	5	4960
ethyl butanoate	fruity	1	410
ethyl 2-methylbutanoate	fruity	0.15	7
methyl 2-methylbutanoate	fruity	0.25	48
acetic acid	sour	60,000	74510
2,3-butanedione	buttery	3	1290
butanoic acid	sweaty, rancid	2730	1790
2-methylbutanoic acid	sweaty	740	2200
ethyl 2-methylpropanoate	fruity	0.1	44
mesifuran	sweet,smoky	25	25

Source. Schieberle and Hofmann(1997).

produced a good estimate of the important aroma-active compounds, but the combination of the two methods could produce a good estimate of the key compounds involved in the aroma of a food. An aroma-recombination study of strawberry was conducted by Schieberle and Hofmann (1997). The authors identified 12 flavor active compounds that when added together in the proper proportions gave the aroma of freshly extracted juice (Table 2.12). The study also evaluated 12 model juices, each with a single component missing. While the authors considered all these compounds to be important, the removal

of DHF or Z-3-hexenal from the mixture caused the most significant change in the overall aroma, indicating that both are character impact aromas in strawberry.

CHAPTER 3
COMPARISON OF EXTRACTION METHODS FOR GAS CHROMATOGRAPHY/OLFACTOMETRY ANALYSIS OF STRAWBERRY FRUIT

Introduction

There are many studies of volatile compounds that require the analysis of large numbers of samples. For example, Kerler (2000) made crosses of 8 cultivars of strawberry and analyzed the fruit of nearly 4500 genotypes for two important volatiles: γ-decalactone and DHF(2,5-dimethyl-4-hydroxy-3(2H)-furanone). The analysis of 4500 samples using solvent extraction was obviously time consuming, but they were able to demonstrate a 6-7 fold increase in the concentration of their selected volatiles in some of the offspring.

It would be advantageous to have a quicker and easier method for analyzing flavor compounds in fruit such as strawberry. Solid-phase microextraction (SPME) is a relatively new technique developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990). It has the advantage of speed, low cost, ease of automation and it does not use organic solvents.

Comparisons between methods are difficult because there are many possible combinations of parameters that make up each method. For SPME, factors such as fiber type, extraction temperature, exposure time, and sample size can all be varied to make an analytical procedure. For solvent extraction, variables such as solvent type, solvent volume, number of extractions, sample size and degree of concentration are important.

Factors such as sample volume, extraction temperature, purge gas rate, mass of adsorbant, and type of adsorbant material are some of the important variables when using dynamic headspace. Since the objective of this study was to look at methods that could be used for large numbers of samples, it was important that the three methods were each optimized based on low cost, speed, simplicity, and small sample size.

With some methods, volatile analysis can be performed on the whole fruit, but more commonly the fruit is homogenized. However, rupturing the cells releases enzymes that can cause major changes in the volatile profile. Buttery et al. (1987) found that $CaCl_2$ was considerably more effective than NaCl in slowing certain enzymatic reactions. The addition of salt to the sample before extraction has also been shown to improve the extraction efficiency for dynamic headspace (Buttery et al., 1987), solvent (Ferreira et al., 1998), and SPME (Yang and Peppard, 1994).

Dynamic Headspace

Tenax is the most common material for dynamic headspace analysis (Nunez et al., 1984). It traps little water, is easy to condition before use and has the highest thermal stability of commonly used adsorbants. Sampling temperatures from ambient (Song et al., 1998; Steffen and Pawliszyn, 1996) to 60°C (Jia et al., 1998; Nonato et al., 2001) have been used for trapping volatiles from fruit or beverages. The use of higher temperatures traps the desired mass of volatiles in a much shorter time, but may also result in the production of artifacts (Sucan et al., 1998).

For quantitative analysis, large traps up to 10 g of Tenax (Buttery et al., 1987) are used, but for flavor profiling, smaller traps from 80 to 120 mg are more common (Buckholz et al., 1980; da Silva and das Neves, 1999; Elmore et al., 1997; Sucan et al., 1998). When using small traps, some of the volatiles will breakthrough and elute from

the trap. However, since dynamic headspace preferentially sweeps the more volatile components into the trap, allowing some of these compounds to pass through the trap can produce a more representative aroma profile (Buckholz 1980). To obtain a representative sample, all the parameters such as sample size, sweep gas flow rate, sample temperature, and collection time must be optimized.

Fortunately, methods for dynamic headspace analysis have previously been reported for strawberries. Shamaila et al. (1992) evaluated volatiles from strawberries stored under a modified atmosphere. Sliced strawberries (300g) were placed in a flask at 40°C and nitrogen was purged through the flask at 30 mL /min for two hours. Volatiles were trapped in a glass tube containing 120 mg of Tenax GC. The trap was eluted with 2 mL diethyl ether and the extract was concentrated to 200 μL with a gentle stream of nitrogen. Da Silva and das Neves (1999) purged 100 g of fresh strawberries held at 45°C for 2 hours using 45 mL/min of nitrogen. Volatiles were swept into a trap consisting of 90 mg of Tenax GC and the trap was directly desorbed into the gas chromatograph using an automatic purge-and-trap injector.

Solvent Extraction

Solvent extraction is another common method for isolation of volatiles. Many methods have been used to study strawberry aroma-active compounds. Larsen and Poll (1995) isolated volatiles from 20 g of strawberry juice with 20 mL diethyl ether/pentane (2:1). Schieberle (1994) extracted volatiles 3 times with diethyl ether (total volume 1.0 L) from 600 g of strawberries homogenized with 600 g saturated $CaCl_2$. Siegmund et al. (2001) obtained an aroma sample from 200 mL of strawberry drink using a single extraction with 20 mL flurotrichloromethane. Lambert et al. (1999) obtained a volatile

isolate from approximately 150 g of strawberry puree by extracting 3 times with 50 mL of dichloromethane.

Aznar et al. (2001) compared Freon 113 with ethyl acetate for the extraction of volatiles from Lychee (*litchi chinesis*) juice stabilized with $CaCl_2$. Ethyl acetate extracted more volatiles than the nonpolar Freon, but the authors felt that a combination of both solvents was needed to obtain a representative sample.

Solid Phase Microextraction

The selection of the fiber coating for SPME analysis is the most important factor governing the amount of analyte extracted (Li and Weber, 1999). The PDMS coated fiber, one of the first commercially available fibers, has been widely used in aroma research. However, PDMS/DVB (introduced in 1996) and PDMS/Carboxen (introduced 1997) are well suited for trace volatile analysis.

Song et al. (1998) compared PDMS and PDMS/DVB coated fiber for selected volatile compounds from tomatoes and strawberries. The partition coefficient for the PDMS/DVB was typically about an order of magnitude higher than that of PDMS for the more polar compounds. Additionally, the range of partition coefficients for PDMS was 120-fold between the compound for which it had the greatest affinity to the compound with the least affinity. In contrast, the range of partition coefficients for the PDMS/DVB was only 3.4-fold. Consequently, the authors suggested that PDMS/DVB extracted a more representative sample and was preferred to PDMS alone. PDMS/carboxen coated fibers are less polar than PDMS/DVB and have a larger proportion of smaller diameter pores. For trace analysis of small molecules, PDMS/carboxen is the best choice.

The maximum amount of volatiles are adsorbed when equilibrium between the sample and the fiber is reached. At ambient temperature, using a wide range of aroma-

active volatiles, Steffen and Pawliszyn (1996) found that it took from 5 to 120 min for equilibrium between the sample and a PDMS coated fiber to be reached and 30 to 60 min for equilibrium using a PA coated fiber. Jia et al. (1998) found that at 40°C the concentration of orange juice volatiles absorbed on a PDMS coated fiber did not increase after 30 min of exposure. Siegmund et al. (2001) monitored changes in the aroma of strawberry drink during storage using a PDMS/carboxen coated fiber. A 10 mL sample was placed in a 40 mL vial and allowed to equilibrate for 10 min at 30°C under constant stirring. The fiber was exposed for 10 min and then transferred directly to the GC injector. The split port was opened after 2 min, but the fiber was left in the injector for reconditioning for the entire 29 min run.

Gas Chromatography/Olfactometry

Gas chromatography/olfactometry (GC/O) describes a system that uses a gas chromatograph to separate volatile compounds from a complex matrix, then uses a human nose as a detector (Blank, 1997). The human nose is extremely sensitive to many aroma-active compounds.

The most common types of GC/O analysis are the dilution methods and the time-intensity methods. Time-intensity methods are based on magnitude estimation of the aroma impact. They have the advantage over dilution methods in that repeated analysis of the sample over a series of dilution is not required. One such method, Osme, has the evaluator rate the strength of the aroma using an electronic time-intensity scaling device (Miranda-Lopez et al., 1992). As originally developed, Osme uses 4 trained observers performing 4 replications each. Peaks detected at least 50% of the time by a given panelist and by at least 3 out of the 4 panelists are than put together to form a consensus Osmegram. Da Silva (1994) evaluated the quantitative capability of Osme using model

solutions of six aroma-active compounds. All four evaluators were able to establish a statistically significant relationship between aroma intensity and concentration for five of the six compounds. Other simpler methods of magnitude estimation are also used. Lesschaeve et al. (1991) used five evaluators to simply rate the intensity of aromas eluting from the chromatograph on a scale from 1 to 4, without the use of electronic recording devices.

One important part of GC/O is the reporting of retention indicies of compounds based on a series of alkane standards rather than retention times (Van Den Dool, 1963). This step is important because retention times of aromas must match up throughout the dilution series and it also links the sensory response to a reproducible chemical property. Since many important aroma-active compounds and their associated retention times (Kovats index) are reported in the literature, calculating the index of an unknown compounds is often a first step in identification.

Objective

The objective of this study was to compare SPME with two other traditional methods of extraction to determine the best method for screening fruit samples for important flavor compounds. SPME was compared to batch solvent extraction and dynamic headspace.

Materials and Methods

Method Comparison Using Gas Chromatography/Olfactometry

Samples of three strawberry varieties (Aromas, Camarosa, and Diamante) were packed in ice and shipped from California by overnight airmail. Approximately 300 g of washed and sliced fruit from each cultivar was blended with 300 mL of a saturated

calcium chloride solution (70 g/100 mL distilled water). Fractions of 30 mL of the puree were measured into separate vials for frozen storage and later extraction and analysis.

Solvent Extraction

Twenty mL of strawberry puree were measured into a 50 mL Teflon centrifuge tube. An aliquot of 10 mL of ethyl acetate was added, and the tube was vigorously shaken for 1 minute. The tube was then centrifuged at 1800 x g for 15 min to break the emulsion. The organic phase was removed and the sample was extracted a second time with another 10 mL of ethyl acetate. The organic phases from each extraction were combined, and approximately 1 g of anhydrous sodium sulfate was added to remove water. An internal standard (50 μg ethyl benzoate) was added and then the eluent was concentrated under nitrogen to 100 μL, and 0.5 μL was injected into the GC.

Dynamic Headspace

The analysis was performed using a method similar to Shamaila et al. (1992). The traps were constructed by placing 100 mg of Tenax TA 60/80 (Supelco, Bellefonte, PA) into a glass disposable Pasteur pipette between two small plugs of salanized glass wool. Twenty-five mL of puree was placed in a 250 mL round bottom flask in a water bath at 40°C. Nitrogen at 40 mL/min was purged through the samples and collected in the traps for 2 hours. The traps were then eluted with 5.0 mL of n-pentane followed by 5.0 mL of ethyl ether. An internal standard (50 μg ethyl benzoate) was added and then the eluent was concentrated under nitrogen to 100 μL and 0.5 μL was injected into the GC.

SPME Extraction

Twenty mL of strawberry puree were measured into a 40 mL SPME vial. An internal standard (50 μg ethyl benzoate) was added and the vial was vigorously shaken for 1 minute. The vial was placed in a 40°C water bath and allowed to equilibrate for 10 min. A manual SPME holder containing a PDMS/carboxen fiber (Supelco, Bellefonte, PA) was inserted into the vial and exposed for 30 min. Preliminary studies showed that headspace exposure times shorter than 30 min did not produce large enough peaks for some of the less volatile compounds such as γ-decalactone to be easily quantified. The fiber was inserted into the injector of the GC and desorbed for 15 min. The purge valve was opened after 5 min, but the fiber remained in the injector to condition it for use in the subsequent sample.

Gas Chromatography/Olfactometry

Chromatography was performed using an HP 5890 series II gas chromatograph (Hewlett Packard Inc., Palo Alto, CA) equipped with a sniffing port (Datu Inc., Geneva, NY). All samples were run on a polar and a non-polar column (Zebron ZB-5 and Zebron ZB-FFAP, Phenomenex, Torrance, CA) and identification of compounds was done by comparing retention times and aroma descriptors with authentic standards on the two columns. Both columns were 30 M x 0.32 mm ID x 0.5 micron film thickness. The temperature program for the ZB-5 column was 34°C to 240°C at 7°/min and for the ZB-FFAP column, 40°C to 240°C at 7°/min. The injector temperature was 240 °C. The column effluent was split, one-third of the flow was routed to the FID and the other two-thirds was mixed with warm humid air and exhausted through the olfactory port for sniffing. Each extract from the three methods was analyzed by GC/O using 2 trained

evaluators in duplicate. Quantification of the olfactory impact was recorded by each sniffer using a slide scale potentiometer. Data from the olfactory input device and the FID were recorded simultaneously using Chrome Perfect software (Justice Laboratory Software, Denville, NJ).

Extraction Efficiency Comparisons

A PDMS/DVB fiber was used in this experiment rather than the PDMS/carboxen fiber used previously. The PDMS/DVB fiber has a more uniform pore size and it produces a more representative sample. Secondly, since it has much lower affinity for methanol, the analysis is not as affected by the addition of the internal standard (dissolved in methanol). Finally, molecules larger than 12 carbon atoms are strongly retained on the carboxen and are difficult to desorb (Mani, 1999). Therefore, the injector temperature needs to be higher, which may result in the formation of artifacts. All other aspects of the methodology were the same as in the first experiment except that the injector temperature was lowered to 200 °C.

The linearity of response and extraction efficiency of the three methods was evaluated by spiking the samples with 5 levels of known standards (propyl butyrate, E-2 hexenal, linalool, hexanoic acid, and γ-decalactone) chosen to represent different chemical classes and a range of peak areas and retention times. The concentration of the standards was chosen for each method so that they were all in the same range of detector response. For example, the concentration of spikes of linalool was 0.04-0.192 ppm for SPME, 0.40-1.2 ppm for solvent extraction, and 0.8 to 4.0 ppm for dynamic headspace.

Results and Discussion

Method Comparison Using Gas Chromatography/Olfactometry

Differences in selectivity between the three extraction methods can be seen in the FID chromatograms in Figure 3.1. The chromatograms clearly show that the solvent extraction is the most efficient method for isolating the later eluting (less volatile) compounds. Major differences in selectivity can also be seen in the early and middle portions of the chromatograms.

The results from the GC/O analysis from the DB-5 column are presented in Table 3.1. A total of 41 compounds were consistently detected by the panelists and given a sensory impact rating between moderate and strong. The results are similar to the results from the FID detector. Results from the later portions of the chromatograms confirm that solvent extraction is superior for isolating the later-eluting compounds. Of the last 10 aroma-active compounds eluting from the column (Table 3.1), only 5 were extracted by SPME and only 2 by dynamic headspace. On the other hand, the olfactory impact ratings were highest with SPME for the earliest 15 compounds eluting from the column. For the earliest eluting peaks, SPME is the method of choice because with the other methods, the compounds are either masked by the solvent front or lost during the concentration step. Of the 41 compounds detected, only 18 could be identified by their aroma and their retention time match with authentic compounds on the ZB-5 and ZB-FFAP columns. This is in agreement with a GC/O study of wine, where Miranda-Lopez et al. (1992) found that over 50% of the aroma-active peaks were not detected by FID. Ten of the 18 identified compounds were quantified using SPME extraction and the method of standard addition, while the other 8 compounds had FID peaks too small to quantify. None of the remaining 23 aroma-active compounds had noticeable FID peaks. Gas Chromatography

was also performed on the three varieties of strawberries using solvent extraction and the ZB-FFAP column (Table 3.2). Only 26 compounds were consistently detected in this analysis compared to the 33 compounds detected using solvent extraction with the ZB-5 column. Acetic acid was the only identified compound that was detected using the ZB-FFAP and not with the ZB-5 column. On the ZB-5 column, acetic acid either eluted with the solvent, or in the case of SPME, the peak was too broad to be detected since organic acids often chromatograph poorly on non-polar columns.

Since all extraction methods are somewhat selective, the sample obtained by any of the three extractions cannot be identical to natural strawberry flavor. Therefore it is important to understand the limitations of the extraction methods used. For example, highly volatile compounds are often not detected because they are lost during the concentration of the extract or are masked in GC/O by the solvent peak. Solid-phase microextraction eliminates the solvent peak, but may still not concentrate highly volatile compounds sufficiently. Grosch (2001) recommends that AEDA analysis be combined with static headspace analysis to produce a more complete picture of the volatile profile. The importance of using both a concentrated extract and static headspace analysis is illustrated by a study of stewed beef juice (Guth and Grosch, 1994). In this study, the two most important aroma compounds determined by AEDA were not important in the static headspace analysis, while the most important compounds determined by static headspace analysis were not listed as important in AEDA.

To see if the three extraction methods studied were missing some important volatile compounds, static headspace extraction was performed. One mL of headspace above a 20 mL sample of strawberry puree held at 40°C was extracted using a gas-tight

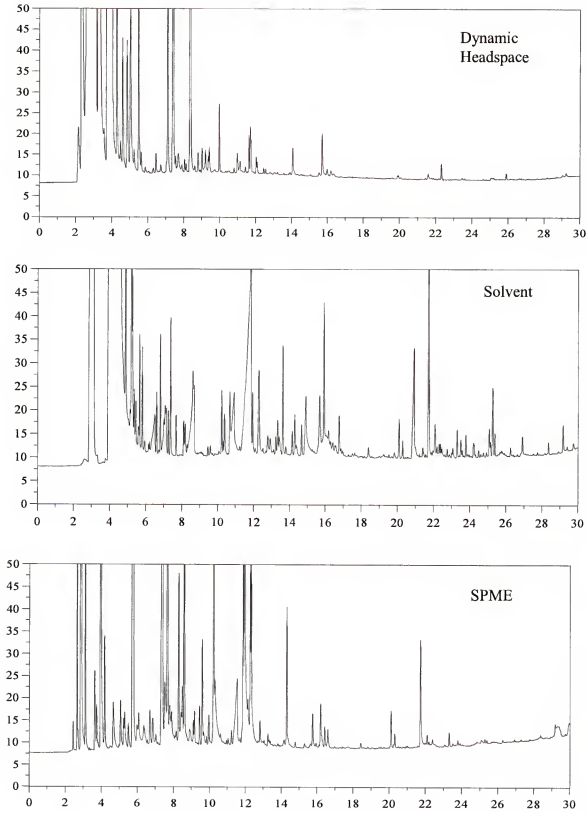


Figure 3.1. Typical FID Chromatograms from the Three Extraction Methods.

Table 3.1. Sensory Descriptors and Tentative Identification of Some Aroma-Active Compounds Detected in Strawberry Fruit and Their Relative Olfactory Impact Measured on an Intensity Scale from 0-100 (Analysis using a ZB-5 Column).

				Relative Olfactory Impact		
Ret Time	Kovats	Descriptor	Compound	Solvent	SPME	P & T
3.65	594	buttery	diacetyl		70	
4.77	668	skunky			46	
5.73	723	fruity/cheese	methyl butyrate		63	36
6.47	760	fruity			46	
6.89	781	fruity	methyl-2-methyl butyrate		52	25
7.32	801	green	hexanal	34	69	41
7.35	802	apple	ethyl butyrate	61	86	63
8.49	850	fruity	ethyl-2-methyl butyrate	a	a	a
8.52	853	fruity	E-2 hexenal	36	54	34
9.03	875	cereal, popcorn		49	48	
9.87	910	cooked potato	methional	44	65	
11.20	966	sweet, floral, fruity		25	39	
11.55	980	green, mushroom	1-octen-3-one	52	75	56
11.65	984	fruity		51	70	47
11.97	998	fruity	ethyl hexanoate	37	48	35
13.02	1043	green olive		32	47	
13.32	1056	caramel	DHF	60	52	38
13.45	1062	musty	mesifuran	65		
14.20	1095	cucumber		50	41	
14.30	1100	lemon	linalool	47	55	43
14.75	1121	stale beer, bread		41		
14.88	1127	green olive		46		
15.35	1149	green		42		
15.50	1156	cucumber, sweet		40	36	
15.70	1165	olive ,sweaty		47		
16.30	1194	mint, rootbeer		55		
16.77	1217	silly putty		50		
16.82	1220	fried, cardboard			54	
17.10	1234	musty, sweaty			46	
17.98	1279	cooked cereal			48	
18.17	1288	mint, clove, spice		45		
18.87	1325	cooked, fried		43		
19.50	1358	rubber		45		
19.72	1370	cinnamon, clove,	eugenol	41	60	
20.27	1400	applesauce	β-damascenone	61	67	45
20.56	1416	chocolate, vanilla	vanillin	61	32	
20.90	1434	olive, buttery		43		
21.40	1462	smokey, burnt		43		
21.70	1479	peach melon	γ-decalactone	49	59	38
22.18	1506	bacon		43		
22.30	1513	coconut, popcorn	δ-decalactone	48	37	

[a] could not be separated from the large peak of E-2 hexenal

syringe. The headspace analysis produced only 3 aroma active peaks, tentatively identified as methyl butyrate, ethyl butyrate and hexanal. No new compounds were detected. One of the problems with using small traps with dynamic headspace is that some of the compounds will breakthrough and elute from the trap (Buckholz et al., 1980). Breakthrough was evaluated by placing 3 traps in series. Each of the three traps contained 100 mg of Tenax TA and the headspace was purged through the traps with nitrogen at 40 mL per min for 2 hours. All three traps and a blank were eluted with pentane/ether and 0.5 μL were injected into the GC. The chromatogram from the first trap contained many peaks, while the second and third traps in the series were identical to the blank, indicating no obvious breakthrough was occurring.

Extraction Efficiency Comparisons

The spiked samples had trends similar to the FID and GC/O analysis in the quantities of compounds extracted by the three methods. Figure 3.2 shows the detector response versus the concentration of E-2 hexenal. For this compound, SPME had the highest recovery and solvent extraction the lowest recovery. The results from the other compounds are presented in Table 3.3.

Linear regression was performed to compare the detector response to the concentration of the compounds. The slope of the regression equation (detector response/concentration) is a measure of the extraction efficiency. High slope values means that the detector response was high compared to the concentration.

For the early eluting compounds; propyl butyrate, E-2-hexenal and linalool, SPME was the most efficient extraction method, followed by dynamic headspace. For

Table 3.2. Sensory Descriptors and Olfactory Ratings of Compounds Detected Using ZB-FFAP Column and Solvent Extraction. Relative Olfactory Impact Was Measured on an Intensity Scale from 0-100.

Ret. Time	Kovats	Descriptor	Compound	Rating
5.15	993	fruity		31
5.34	1005	buttery	diacetyl	68
6.14	1055	fruity, apple	ethyl butyrate	58
6.62	1084	green fruit		27
8.05	1164	green apple		30
9.66	1250	fruity	ethyl hexanoate	31
11.07	1324	mushroom	1-octen-3-one	60
13.21	1439	mint		57
13.72	1470	sour	acetic acid	51
14.15	1491	cooked potato	methional	49
14.89	1533	cucumber		45
15.37	1561	lemon	linalool	54
16.52	1629	musty, cocoa	mesifuran	48
16.83	1648	sweaty		56
17.57	1692	rubber		51
20.12	1858	applesauce	β-damascenone	46
20.38	1872	green, musty		44
20.80	1900	green olive		47
21.81	1970	coconut		40
23.18	2069	caramel	DHF	52
25.09	2221	clove	eugenol	44
25.66	2271	coconut, popcorn		51
27.45	2442	burnt match		31
27.82	2481	wet sponge		45
28.15	2517	green olive		51
30.13	2764	vanilla		65

the later eluting compounds (hexanoic acid and γ-decalactone), the solvent extraction was the most efficient followed by SPME. The dynamic headspace analysis resulted in recovery rates too low to analyze as shown by the very low regression coefficient. Overall, the solvent extraction method produced relatively equal extraction efficiencies for all compounds, while SPME had very high recovery rates for propyl butyrate and linalool. Yang and Peppard (1994) also noted high recovery rates for linalool when using a PDMS coated fiber. The extraction efficiency of dynamic headspace was so low for hexanoic acid and γ-decalactone, that there was no significant detector response to the spikes.

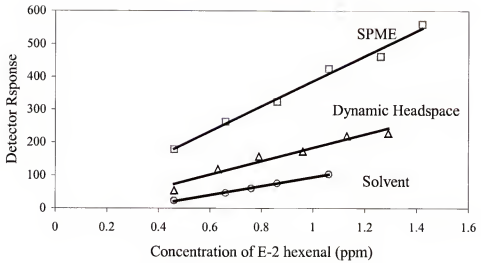


Figure 3.2. Comparison of the Three Extraction Methods for Extracting E-2 Hexenal from Strawberry Puree.

The linearity of response as indicated by the R^2 value was better with SPME than the other methods for all 5 compounds evaluated. Even excluding the extremely low R^2 values caused by the poor extraction efficiency of dynamic headspace for hexanoic acid and γ-decalactone, this method was not as precise as the other two methods.

Table 3.3. Detector Response Versus Concentration in Strawberry Puree Spiked with Selected Flavor Compounds.

	SPME		Solvent		Dynamic headspace	
Analyte	Slope	R^2	Slope	R^2	Slope	R^2
Propyl Butyrate	1680	.99	130	.99	212	.92
E-2 Hexenal	383	.99	136	.99	204	.95
Linalool	1965	.99	136	.95	158	.88
Hexanoic Acid	152	.99	268	.99	7	.19
γ-decalactone	146	.98	333	.97	6	.29

Conclusion

Because of their very low aroma thresholds, many of the compounds with olfactory impact have an extremely small FID response. One of the main problems with

all of these methods was that the FID peaks for many of the aroma-active compounds were too small to quantify, or in some cases, even to identify the compounds. For these compounds, it may be necessary to further concentrate the sample before analysis.

While none of the methods were able to identify and quantify a majority of the aroma-active compounds, these methods could still be used to analyze many important volatile compounds. Of the methods tested, SPME proved to be the best method for screening strawberry samples. It could be used for quantifying many of the important aroma-active esters in strawberry fruit. Methyl butyrate, ethyl butyrate, methyl-2-methyl butyrate, ethyl-2-methyl butyrate, methyl hexanoate, and ethyl hexanoate could all be quantified using SPME and a FFA column.

Strawberry breeding programs could benefit from the use of SPME coupled with gas chromatography to evaluate the ester content of the fruit which are quantitatively and qualitatively the most important volatiles in strawberries (Latrasse, 1991). Strawberries differ markedly in their ester profile and breeding schemes crossing varieties with contrasting ester profiles could result in increased overall ester content of the fruit.

Other important compounds such as diacetyl, E-2 hexanal, hexanal, linalool, and γ-decalactone could also be quantified with SPME, and monitoring these compounds could also be part of a breeding program.

CHAPTER 4
RELATIONSHIPS BETWEEN SENSORY EVALUATION AND VOLATILE COMPONENTS OF FIVE STRAWBERRY VARIETIES

Introduction

Strawberry aroma has been studied for more than 40 years and over 360 volatiles have been identified. Of the many compounds listed for strawberries, it is not clear which are the most important. Sensory analysis provides important information on the sensory characteristics of fruit but it is time consuming, and variability between panelists can be high. It is too difficult to use for routine screening of strawberry varieties in a plant-breeding program. Additionally, sensory analysis does not provide a reason for flavor differences detected between samples. In order to understand the causes of the differences, sensory evaluation must be correlated with chemical analysis of the fruit. It would be advantageous to have an instrumental method to analyze fruit and measure the individual components that are the most important to the sensory quality of the fruit. However, if instrumental analysis is to be used to predict sensory quality, chemical analyses must first be correlated with sensory characteristics.

Chemical Analysis

The first step in chemical analysis is to isolate a representative sample from the fruit matrix. Solid-phase microextraction (SPME) is a relatively new technique developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990). This technique, which uses a polymer coated silica fiber to extract and concentrate volatiles from the headspace over a sample, and has the advantages of speed, low cost, and ease of

automation. SPME has been used by Golaszewski et al. (1998), Ibanez et al. (1998), Siegmund et al. (2001), and Ulrich (1997) to study strawberry flavor.

Another important analytical tool is gas chromatography/olfactometry (GC/O). This analytical method uses a gas chromatograph to separate volatile compounds from a complex matrix, and then uses a human nose as a detector (Blank, 1997). The use of a human subject in GC/O provides an important bridge between sensory and chemical analysis. The human senses rather than the impact on an electronic detector is measured, and the most important aroma-active compounds can be selected from the many volatiles present.

One of the many methods developed for GC/O is called Osme. It uses an evaluator to rate the strength of the aroma eluting from the column using an electronic time-intensity scaling device (Miranda-Lopez et al., 1992). As originally developed, Osme uses 4 trained observers performing 4 replications each. Peaks detected at least 50% of the time by a given panelist and by at least 3 of the 4 panelists are considered important aroma-active compounds. Other simpler methods of magnitude estimation are also used. In a study of strawberry jam, Lesschaeve et al. (1991) used five evaluators to simply rate the intensity of aromas eluting from the chromatograph on a scale from 1 to 4, without the use of electronic recording devices.

The complexity of the food matrix and the wide range of chemical compounds with aroma activity make obtaining a representative sample difficult. For this reason, gas chromatography/olfactometry or other analytical data may not correlate well with sensory data. Instrumental analysis is also difficult because many compounds responsible for the overall flavor of foods are often below the detection limit of the instrument. This often

leads the scientist correlating sensory data with instrumental measurements of only those substances that can be easily measured, regardless of their aroma activity.

Sensory Analysis

Sensory analysis of aroma is difficult because of problems such as the interference from taste and trigeminal responses, poor memory of intensity of aromas, and carryover (where one sample affects the evaluation of subsequent samples). In spite of these difficulties, descriptive analysis provides a relatively complete evaluation of the important parameters contributing to the difference between samples.

Quantitative Flavor Profiling (QFP) is one of many descriptive techniques that have been developed (Stampanoni, 1993). This method uses a group of experienced flavorists to generate descriptive terms prior to the actual evaluation. This reduces the training time and has the advantage that the panelists do not have any preconceived ideas or biases since they are not involved in the development of the descriptors (Stampanoni, 1993).

In spite of these difficulties, correlations between sensory and instrumental analysis has been the subject of many studies (Boccorh et al., 1999; da Silva and das Neves, 1997;Togari et al.,1995). Principal component analysis (PCA) or partial least squares regression analysis (PLS) are two of the most commonly used methods to analyze data from these studies.

Objectives

The objectives of this study were to identify the most important volatiles in 5 varieties of strawberries commonly grown in Florida, and to correlate the concentration of aroma-active compounds with sensory ratings for the five varieties.

Materials and Methods

Sensory Evaluation

Sixteen panelists, 10 men and 6 women between the ages of 21 and 43, were trained using fresh fruit, reference standards, and strawberry puree spiked with reference standards. Flavor descriptors and reference standards were selected by the panel leader prior to training and were based on strawberry descriptors found in the literature (Stampanoni, 1997; Ulrich et al., 1997), and previous GC/O analysis of fresh strawberry fruit. Reference standards for the descriptors were made up in propylene glycol as follows: green (1% hexanal), fruity (1% ethyl hexanoate and 0.1% ethyl-2-methyl butyrate), floral (1% linalool), caramel (0.1% DHF (2,5-dimethy-4-hydroxy-3(2H)-funanone)) and peach (1% γ-decalactone). In addition to the aroma descriptors, the panelists were asked to rate sweetness, sourness, strawberry flavor intensity and overall acceptability.

In the first training session, panelists were presented with fresh strawberry puree spiked with the standard references and were asked to describe the differences between the samples. The sample spiked with DHF, was generally rated as more sweet, jam like and cooked. The linalool sample was rated as more fresh and less jam like. The reference for green was presented as a mix of hexanal and E-2 hexenol, and was described as chemical, unpleasant, and solvent. These descriptors were attributed to the high level of E-2 hexenol and this compound was removed from the reference and for the rest of the study, hexanal alone was used as the green reference standard. The fruity standard was spiked with methyl butyrate and this sample was described as apricot, candy, or strawberry gum. Generally it was agreed that ethyl butyrate was not a good representation of the fruity quality of strawberry. After further experimentation in the lab

with an informal panel, it was decided that a mix of ethyl hexanoate and ethyl-2-methyl butyrate provided a better reference standard and was used throughout the remainder of the study. The descriptor peach was represented by γ-decalactone, and was described as lotion, dusty, or artificial by the panelists. In order for the panelists to get a better understanding of the peach descriptor, they were presented with a pure sample of γ-decalactone instead of the spiked strawberry puree. Following the evaluation of the samples, the panelists were informed as to the identity of the reference samples and the descriptor that should be used with each of the references. The panel was then provided with 2 samples of strawberries and were trained in the use of a draft ballot. In the next 3 training sessions the panelists were presented with strawberry puree spiked with reference standards and were asked to provide the descriptor that best fit the spiked sample. Panelists who did not identify the spiked samples correctly were given further training. The panelists were then presented with the group of reference standards and 3 samples of fresh fruit differing as much as possible in the main attributes. The panelists were asked to smell the standards to refresh their memory as to the aroma of the selected attributes and then to practice rating the samples.

The procedures used in the next 2 training session were exactly as the procedures used during the actual analysis of the strawberry varieties. The sensory analysis was performed in private booths. Each panelist was presented with the reference standards, water and an unsalted cracker to cleanse the palate and five samples of fresh chopped strawberry fruit. Sample presentations were chosen so that each cultivar was presented at each position in the order, an equal number of times. Approximately 100 fruit from each of the 5 varieties were washed, finely chopped, mixed together, and placed in 118 mL

plastic cups with lids. Results from these 2 trial runs were analyzed to assess the performance of the panelists.

For the experiment, data were collected on 4 commercial cultivars commonly grown in Florida (Camarosa, Mirador, Strawberry Festival, and Sweet Charlie) and one experimental line designated FL96-114. Fruit was harvested near Plant City, FL, and transported to Gainesville, FL where it was stored under refrigeration (2°C) prior to analysis. Sensory analysis was conducted the following day, and a second replication was conducted 2 days later.

For GC/O and chemical analysis, samples of fruit from each cultivar were blended with an equal volume of saturated calcium chloride solution (70 g/100 mL distilled water), and placed in 30 ml glass vials for frozen storage and later extraction and analysis.

SPME Extraction

Twenty mL of strawberry puree from one of the varieties was measured into a 40 mL headspace vial. An internal standard (50 μg ethyl benzoate) was added and the vial was vigorously shaken for 1 min. The vial was placed in a 40°C water bath and allowed to equilibrate for 10 min. A manual SPME holder containing a polydimethylsiloxane/ divinylbenzene (PDMS/DVB) fiber (Supelco, Bellefonte, PA) was inserted into the vial and exposed for 30 min. The fiber was then inserted into the injector of the GC and desorbed for 15 min. The purge valve was opened after 5 min, but the fiber remained in the injector to condition it for use in the subsequent sample. Four replications were run for each cultivar on each of two different GC columns.

Gas Chromatography/Olfactometry

Chromatography was performed using an HP 5890 series II gas chromatograph (Hewlett Packard Inc., Palo Alto, CA) equipped with a sniffing port (Datu Inc., Geneva, NY). All samples were run on a polar and a non-polar column (Zebron ZB-5 and Zebron ZB-FFAP, Phenomenex, Torrance, CA) and identification of compounds was done by comparing retention times and aroma descriptors with authentic standards on the two columns. Both columns were 30 M x 0.32 mm ID x 0.5 micron film thickness. The temperature program for both columns was 40°C to 240°C at 8 degrees/min. The injector temperature was 240°C. The column effluent was split, one-third of the flow was routed to the FID, and the other two-thirds was mixed with warm humid air and exhausted through the olfactory port for sniffing. Samples from each of the 5 varieties were analyzed by GC/O using 2 trained evaluators in duplicate. Quantification of the olfactory impact was recorded by each evaluator using a slide scale potentiometer. Data from the olfactory input device and the FID were recorded simultaneously using Chrome Perfect software (Justice Laboratory Software, Denville, NJ).

Chemical Analysis

Some of the most important volatiles determined by GC/O were quantified in the fruit using the method of standard addition. A sample of strawberry puree was spiked with 4 levels of known concentrations of authentic standards and standard curves were developed using linear regression analysis using Excel (Microsoft Corp., Redmond, WA).

Sugar content was measured using a Reichert Mark II Refractometer (Warner-Lambert Tech. Inc., Buffalo, NY). Ten representative fruit were brought to room temperature, washed, the calyx was removed, and the fruit was homogenized in a blender for 1 min. A sample of puree was pipetted onto the prism of the refractometer and the

brix was recorded in the brix-temperature mode. Three replications were measured for each cultivar.

Titratable acidity was measured using Accumet Basic AB 15 pH meter (Fisher Scientific, Pittsburgh, PA). Strawberry puree (10 g) was mixed with 90 mL of distilled water and the sample was titrated with 0.1 N NaOH to pH 8.2. Results from the average of 2 replications are expressed as g/100 mL citric acid.

Results and Discussion

Differences in the descriptive ratings for the strawberry varieties were analyzed using ANOVA (SAS, Version 8.2, SAS Institute, Cary, NC). A randomized complete block design was used, with varieties as treatment and panelists as replications. Duncan's Multiple Range Test was used to separate means if a significant difference ($p = 0.05$) was indicated by ANOVA. Simple correlations between all measurements (sensory ratings, GC/O ratings, and chemical composition) were also calculated. Significant differences in panelists ratings were found for all descriptors, but there was no significant panelist*cultivar interaction, indicating that the panelists were rating the samples in the same way, but were using different parts of the rating scale. Significant differences between varieties were found for all of the descriptors (Table 4.1). Sweet

Table 4.1. Sensory Ratings for the Flavor Attributes for the Five Strawberry Varieties.

Cultivar	Strawberry	Green	Fruity	Floral	Caramel	Peach
Camarosa	55 c	64 ab	45 c	49 c	35 bc	50 c
S. Festival	85 b	52 b	66 b	74 a	40 bc	68 ab
Mirador	75 b	60 ab	58 bc	56 bc	45 ab	47 c
Sweet Charlie	102 a	33 c	94 a	71 ab	58 a	78 a
V 96-114	62 c	74 a	47 c	65 abc	30 c	60 bc

Sensory ratings range from Low (0) to High (160). Means followed by the same letter are not significantly different at the 5% level.

Charlie was rated significantly higher than the other varieties for sweetness, strawberry flavor intensity, fruity, caramel, and peach. It was also rated significantly lower than the other varieties for green and sourness. The sweetness and sourness ratings agree with the chemical analysis of Sweet Charlie which had the highest soluble solids content and the lowest titratable acidity. The variety FL96-114 had the highest acidity and it also ranked highest for green and sourness. This variety was rated significantly less sweet than the other varieties even though it had an intermediate level of sugar. Camarosa had the lowest ratings for strawberry flavor intensity, fruity, and floral and ranked next to lowest for caramel and peach. The Camarosa sample evaluated in this test was of lower quality than is typical for this popular cultivar.

Table 4.2. Sensory Ratings for the Taste (Sweet and Sour) Attributes and Analysis of Brix and Titratable Acidity for the Five Strawberry Varieties.

Cultivar	Sweetness	Sourness	Brix	Acidity
Camarosa	40 c	86 a	5.90 d	0.768 b
S. Festival	76 b	49 bc	6.40 c	0.752 b
Mirador	65 b	62 b	4.85 e	0.723 b
Sweet Charlie	95 a	38 c	7.30 a	0.560 c
V 96-114	33 c	100 a	6.65 b	0.877 a

Sweetness and Sourness range from Low (0) to High (160). Means followed by the same letter are not significantly different at the 5% level.

In addition to the analysis of variance, the aroma attributes were analyzed by principal component analysis using The Unscrambler v 7.6 (CAMO ASA, Oslo, Norway). The descriptors green and sour, sweet and strawberry flavor intensity, and floral and peach, grouped together on the loading plot indicating that these 3 pairs of descriptors were related (Figure 4.1). The grouping of sour and green may mean that some of the varieties were not quite ripe since these attributes usually describe immature fruit. It is not surprising that strawberry flavor intensity was grouped together with

sweet, since sweetness is known to be an important flavor enhancer (McBride, 1990). It is not clear why the peach and floral attributed loaded together on the PCA plot. It is also interesting to note that floral, peach and caramel, and strawberry, fruity and sweetness loaded together on the first principal component (x-axis).

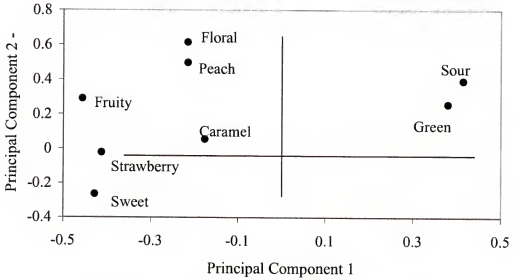


Figure 4.1. Loading Plot for the Strawberry Aroma Descriptors on the Plane Described by the First and Second Principal Components.

There were 17 compounds consistently detected by GC/O. This is considerably less than the number of compounds detected previously in other studies. This may be because previous studies used PDMS/carboxen coated SPME fibers which generally are more efficient at extracting the lower molecular weight volatiles. On average, the 5 compounds with the highest GC/O ratings were, E-2-hexenal, γ-decalactone, linalool, DHF and ethyl butyrate. The surprising thing about these aromas being rated the most intense is that their descriptors are; green-fruit, peach, floral, caramel, and fruity, almost the same descriptors as used for the sensory panel. In contrast to the results from the descriptive analysis, significant differences between varieties were found for only 3

Table 4.3. Sensory Ratings and Identification or Aroma Descriptor of Compounds Detected in the Strawberry Fruit using Gas Chromatography/Olfactometry.

		Sensory Rating of Aroma Impact (Scale = 0-100)				
Compound or Descriptor	Kovats	CAM.	Fes.	Mir	Sw Ch	FL96-114
'skunky'	665	3	23	31	37	27
methyl butyrate	724	41	36	15	40	17
'fruity'	765	35	24	20	12	19
methyl-2-methyl butyrate	784	47	41	30	33	38
hexanal	804	69	32	43	31	50
ethyl butyrate	807	50	61	45	69	69
E-2 hexenal	856	58	50	50	43	51
'fruity'	965	9	21	7	7	15
1-octen-3-one	978	64	63	54	53	53
'green fruit'	984	50	48	33	60	48
ethyl hexanoate	997	0	0	0	52	41
DHF	1060	62	39	41	47	21
linalool	1100	54	54	62	67	56
β-damascenone	1401	52	60	49	69	68
γ-decalactone	1482	0	0	0	68	0
'floral'	1504	0	25	0	40	0
γ-dodecalactone	1693	46	59	0	49	55

of the aroma-active compounds using GC/O. This was due both to the small differences in the means of the rating for the compounds and the high standard deviation among the ratings.

Models to correlate sensory and chemical analyses were constructed where possible for each of the descriptors. Results from the simple correlations and the PCA analysis was used to judge what compounds should be used in the models.

Simple linear regression was performed between the panelist rating for sourness and titratable acidity. This simple model fit the data fairly well (Table 4.4). The level of sweetness as measured by brix was added to the model. McBride and Johnson (1987) showed that perceived sourness could also be affected by sugar content. Therefore, another model was constructed taking brix into account. This model was not an improvement to the simple model based solely on titratable acidity.

Table 4.4. Models Used to Describe the Sourness of Strawberry.

Model	R^2	Pr > F
Sour = 193.9*acidity – 75.7	.745	0.060
Sour = 1.123*brix + 196.5*acidity –84.5	.746	0.254

The sweetness rating of a product such as strawberry fruit is not only affected by the amount of sugar, but also by its acidity and flavor. In an evaluation of the sweetness of orange juice, Gordon (1965) showed that the sweetness rating could be described by the equation: Sweetness = X_2*sugar conc. + X_1*acid concentration + X_0 (where X_0,X_1,and X_2 are constants). The author also reported that the linear terms were significant but the quadratic and interactions terms were not significant. Stevenson, et al. (1999) also showed that The rated sweetness of a sucrose/citric acid base was affected by the addition of a flavorant. Food flavors such as strawberry or caramel tended to increase the rated sweetness, while non-food aromas such as damascone or eucalyptol tended to decrease the rated sweetness. Since sweetness has been show to be affected by sourness and flavor, a model to explain the sweetness rating should contain these additional factors.

Three models to estimate sweetness are presented in Table 4.5. Estimates from the models were regressed against the sweetness ratings to produce the R^2 values. The first model, using only brix to estimate sweetness did not produce estimates close to the sweetness ratings. The model was considerably improved by taking the acidity of the fruit into account. A third model, including an estimate of the overall aroma of the fruit was also evaluated. The overall aroma was estimated using a model for the intensity of aroma mixtures (Laffort and Dravnieks, 1982). In this model, the total aroma intensity is

equal to $(A^2 + B^2+C^2 \ldots\ldots +X^2)^{1/2}$ where A, B, C, and X represent the aroma intensity of each of the components.

While this model had a better fit, its probability was lower, indicating that it was not a better model than the brix plus acidity model. In addition, the coefficient for the flavor component was small, indicating that the flavor component had at most a small effect on perceived sweetness. One final model using only the brix/acid ratio had the best fit of all the models, but it the relationship was still not significant based on the p value of 0.154.

Table 4.5. Models Used to Describe the Sweetness of Strawberry.

Model	R^2	Pr > F
Sweet = 8.51*(brix) + 8.87	0.093	0.62
Sweet =17.87*(brix) –68.77*(acidity)	0.357	0.02
Sweet = 16.45*(brix) – 70.42*(acidity) + 0.059*(flavor)	0.356	0.12
Sweet = 7.54*(brix/acid) – 3.849	0.545	0.154

At least 131 esters have been reported in strawberry and they largely dominate the total volatiles both qualitatively and quantitatively (Latrasse, 1991). Methyl butyrate, ethyl butyrate, ethyl–2-methyl butyrate, methyl hexanoate, and hexyl acetate, and E-2-hexenyl acetate have been reported as the most important esters in strawberries (Latrasse, 1991; Schieberle and Hofmann, 1997). These compounds have generally been described as having fruity character. Six compounds with fruity aroma were consistently detected by GC/O. Four of these compounds were identified as ethyl butyrate, methyl butyrate, methyl-2-methyl butyrate and ethyl hexanoate and the other two compounds were not identified. An additional compound, methyl hexanoate, was detected by the FID, but it was not consistently reported by GC/O. Hexyl acetate and E-2-hexenyl acetate were not detected in any of the 5 varieties. Five of the esters were quantified and the aroma impact

of the compounds was estimated by calculating the square root of the aroma value (Table 4.6). The combined sensory impact of the esters was then estimated using a model for the overall intensity of aroma mixtures described previously (Laffort and Dravnieks, 1982).

Tale 4.6. Aroma Values and Estimated Overall Intensity of the Five Esters Measured in the Five Varieties of Strawberries.

Cultivar	EB	MB	M2MB	EH	MH	Intensity
Camarosa	20.25 a	7.17 b	2.10 a	0.00 d	1.57 c	23.0
S. Festival	17.61 b	9.47 a	1.79 b	3.61 c	1.65 c	23.1
Mirador	12.65 c	5.00 c	1.79 b	0.00 d	1.41 c	14.8
Sweet Charlie	17.89 b	7.86 b	2.19 a	6.32 a	2.79 a	23.5
FL96-114	17.61 b	6.06 c	1.55 b	5.48 b	2.22 b	21.5

Means followed by the same letter are not significantly different at the 5% level.
EB = ethyl butyrate, MB = methyl butyrate, M2MB = methyl-2-methyl butyrate, EH = ethyl hexanoate and MH = methyl hexanoate.

Several models were used to correlate the estimated sensory impact of the esters (esters) with the fruity rating for the strawberry varieties (Table 4.7). In the first model, the fruity ratings were regressed directly against the esters, producing a model with a low regression coefficient and a low probability of a significant relationship. The second model compares the sensory score with the esters plus brix. Sugar content has been shown to influence flavor ratings. In fact, the addition of sugar to a model drink base can increase the perceived flavor intensity more than the addition of a flavorant (McBride, 1990).

McBride and Johnson (1987) and Gordon (1965) have shown that flavor intensity is also affected by the acidity level. Therefore, two additional models were evaluated using the brix-acid ratio to represent the influence of both sugar and acid on the intensity of fruity flavor. The model using brix/acid and esters had a better fit, but a lower

Table 4.7. Models Used to Describe the Fruitiness of Strawberry.

Model	R^2	Pr > F
Fruity = 1.35*(esters) + 33.45	0.060	0.69
Fruity = 24.08*(brix) –3.70*(esters) – 9.44	0.445	0.56
Fruity = 8.76*(brix/acid) – 1.98*(esters) –27.61	0.915	0.08
Fruity = 7.17*(brix/acid) – 0.43	0.822	0.03

probability of significance than the model using brix/acid alone. In any case, the negative coefficient for the esters indicated that the influence of this term is small at best. Since the compound γ-decalactone was highly correlated ($p< 0.05$) with the fruity rating, this compound (which is an ester) was added to the ester term in the model. This addition improved the model only slightly (data not shown).

The compound DHF (2-5-dimethyl, 4-hydroxy, 3(2H) furanone) has often been reported to be the most important volatile in strawberry (Schieberle and Hofmann, 1997; Ulrich et al., 1997). It has an aroma often described as caramel or cotton candy. It is interesting to note that a commercial caramel flavor (not specifically DHF) was shown to significantly enhance the perceived sweetness of a sucrose solution and to suppress the perceived sourness of a citric acid solution (Stevenson et al., 1999). This effect was higher for the caramel flavor than for any another flavor tested.

Significant differences between varieties were found for the GC/O ratings for DHF, but this compound was not quantified because the FID peak was too small. The GC/O ratings for DHF were significantly correlated with the green descriptor. However, it is probably not the case that DHF influences the green rating, it is more likely that this compound just happened to be higher in the varieties that had higher sensory score for green. The sensory ratings for caramel were significantly correlated with the sensory

ratings for sweet and fruity and negatively correlated with sour. Again, it could not be determined if DHF had any influence on the sweet, fruity, or sour ratings.

Two compounds with the aroma of peach, γ-decalactone, and γ-dodecalactone were detected in some of the varieties but not in others. The concentration of γ-decalactone was measured, but the FID peak for γ-dodecalactone was too small for quantification. There was relatively good agreement between the sensory ratings for peach and the chemical analyses. Sweet Charlie contained both compounds and was given the highest rating for peach by the panelists. Conversely, neither compound was detected in Mirador, and this strawberry cultivar received the lowest rating for peach. The sensory ratings for peach were not significantly correlated with any of the other sensory ratings.

The most important compounds that give a green note have been reported to be hexanal, Z-3 hexenal, and Z-3-hexen-1-ol (Ulrich et al., 1997; Latrasse, 1991). Gas chromatography resulted in only one compound (hexanal) with a green descriptor. Hexanal was also measured in the puree. There was no correlation between the GC/O ratings for hexanal and the green sensory descriptor, nor was there a correlation between the concentration of hexanal in the puree and the green rating. There was also no correlation between the GC/O rating for hexanal and the concentration of hexanal. Because hexanal was the only compound with a green descriptor, and it was not correlated with the green sensory rating, no model for the green could be developed.

The sensory rating for green was positively correlated with the rating for sour and negatively correlated with the ratings for fruity, caramel and sweet. The correlation of

green and sour may indicate that the varieties Camarosa and FL96-114 (which had the highest scores for green and sour) were somewhat immature when evaluated.

Linalool and an unknown compound with a retention index of 1504 on the DB-5 column were the only compounds with a floral descriptor detected by GC/O. Neither compound was significantly correlated with the sensory ratings for floral. Additionally, the sensory ratings for floral were not correlated with any of the other sensory ratings.

Conclusions

In general, it was found to be very difficult to correlate sensory and instrumental ratings. Part of the problem stems from the high variability of the sensory and GC/O ratings. Significant differences between varieties was found for only 3 of the 17 compounds evaluated by GC/O, and the significance of 2 of these compounds was found only because they were not detected in all of the varieties. No correlation between the aroma descriptors and the chemical analysis could be observed except for the peach descriptor. The fact that the two compounds with peach aroma were not found in all varieties made it possible to relate the sensory rating for peach with the chemical analysis.

Flavor mixes are very complex, and even if the aroma-active components are known it may not be possible to predict the sensory response. Kendall and Neilson (1966) found that a simple mixture of methyl salicylate and anethol produced a floral aroma that was uncommon to either of the two components. This blending of aroma may be one of the reasons that correlations between chemical and sensory analyses were difficult to develop.

Kendall and Neilson (1966) found that a difference in concentration of 60% was required for a just noticeable difference between samples of geranial. The task of

determining differences between concentrations of the various compounds in the five varieties of strawberries would be a considerably more difficult task. The fact that it takes fairly large differences in the concentration of compounds to produce sensory differences is another reason correlating instrumental and sensory analyses is difficult. The differences in the levels of aroma compounds among the 5 varieties was probably too small to be discerned by sensory analyses, except in the cases where some compounds were absent from some of the varieties.

CHAPTER 5
CHANGES IN VOLATILE SULFUR COMPOUNDS IN STRAWBERRIES DURING HEATING

Introduction

Although sulfur compounds usually represent a small proportion of total volatiles, they are important aroma-active compounds in fruits such as durian (Weenen et al., 1996), grapefruit (Buettner and Schieberle, 2001), melon (Wyllie and Leach, 1992), and tomato (Buttery et al., 1987). In strawberries, Dirinck et al. (1981) reported the presence of methanethiol, dimethyl sulfide, dimethyl disulfide, methyl thioacetate, and methyl thiobutyrate. They determined that these compounds, especially methanethiol, are important contributors to the aroma of some varieties.

Heating of strawberry puree causes significant changes in the flavor. Sloan (1969) detected dimethyl sulfide, aceteldehyde, isobutyraldehylde, furan, 2-furaldehyde, 2-acetyl furan, and ethyl furoate in strawberry puree heated at 120 °C for 30 min. Dimethyl sulfide was not found in the unheated puree and increased to 0.44 ppm after 10 min. Further heating did not significantly increase dimethyl sulfide.

Solid-phase microextraction (SPME) is a relatively new technique developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990). This technique, which uses a polymer coated silica fiber to extract and concentrate volatiles from the headspace over a sample, has the advantages of speed, low cost, and ease of automation. Additional advantages of SPME are that losses of trace volatiles by adsorption to large surfaces is minimized, and there is no solvent peak to interfere with the detection of early eluting

compounds (Pelusio et al., 1995). Solid-phase microextraction has been successfully used to study sulfur volatiles in wine (Mestres et al., 1999), truffles (Pelusio et al., 1995), and butter (Shooter et al., 1999).

Objectives

The objectives of this research were to determine the major sulfur volatiles in strawberries, to quantify their changes during heating, and to estimate the sensory impact of the changes of the sulfur compounds.

Materials and Methods

Sample Preparation

Strawberries (*Fragaria x ananassa*) were obtained from a local supermarket, and were washed and then macerated for one minute in a blender. The puree was filtered through a double layer of cheesecloth and placed on ice. Approximately 6 g of the puree were pipetted into 3 thin-walled Pyrex NMR sample tubes (17.8 cm x 5 mm OD) for rapid uniform heating. The tubes were heated in a water bath at 95°C for 0.5, 1.0, 1.5, 2.5, 4, or 10 minutes and then cooled in an ice water bath for 1 min. After cooling, 5.0 g of puree was removed from the tubes and placed in an amber colored 40 mL SPME vial with a Teflon coated septa. Propyl thiocyanate (internal standard) and 5.0 mL of saturated calcium chloride were added and the vial was shaken for 30 sec to mix the solutions. The vials were then placed at -10 °C and held until analysis 2 days later. Preliminary tests showed the concentration of many sulfur compounds were above the detector limit, so the samples were further diluted 5 fold with saturated calcium chloride before analysis. The diluted samples were placed in a 40°C water bath and allowed to equilibrate for 45 min. A SPME sampler was then injected through the septa and the fiber (PDMS/Divinyl benzene) was exposed to the headspace for 10 min.

For the analysis of fresh fruit, ten representative fruit with minimal bruising were placed in a 500 mL beaker and the top was sealed with aluminum foil. After 10 min, the SPME fiber holder was inserted through the foil and the fiber was exposed to the headspace for an additional 10 min.

GC/Sulfur

The SPME fiber was desorbed in the injector of an HP 5890 Series II at 200°C for 5 min using splitless mode. Compounds were separated on 30 M x 0.32 mm ID x 0.5 mm (5% phenyl)-methylpolysiloxane column (J & W Scientific Inc., Folsom, CA). The injector temperature was 200°C and the temperate program was 40°C to 250°C at 8°C/min. Compounds were analyzed using a Model 5380 Pulsed Flame Photometric Detector (OI Corporation, College Station, TX). Compounds were identified by comparing retention times with authentic standards on the DB-5 column and on a 30 M x 0.32 mm ID GS-GASPRO column (J & W Scientific Inc., Folsom, CA). The temperature program for the GS-GASPRO column was 200°C to 250°C at 2.5°C/min with a 10 min hold at 250 °C.

Quantification of Sulfur Compounds

Since the quantities of volatiles extracted by SPME are dependent on the sample matrix, standard curves were made up in strawberry puree diluted with the same amount of saturated calcium chloride as the samples. Prior to the addition of the standards, the background sulfur compounds were removed from the puree by vacuum stripping for 1 hour at 40°C using a Rotavapor (Brinkman Instruments, Westbury, NY).

Results and Discussion

Carbon disulfide, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, methyl thioacetate, and methyl thiobutyrate were identified and quantified in the strawberry

puree. Sulfur dioxide, hydrogen sulfide, methanethiol, and methional were also identified but could not be quantified. Aroma values for the compounds that could be quantified were calculated by dividing the concentration of the compound by its threshold concentration. Aroma values of 1 or greater mean that the concentration of the compound exceeds its threshold concentration and therefore it could have sensory impact. The higher the aroma value, the more the compound should contribute to the overall aroma. However, the aroma values calculated are based on average values published in the literature and are from many different authors.

Table 5.1. Thresholds and Aroma Values for Sulfur Compounds in Fresh Strawberry Fruit, Puree, and Puree Heated for 10 min at 95 °C. Threshold Values are Calculated by Dividing Concentration by the Threshold Value.

		Aroma Values		
mpound	Threshold (ppb)	Fresh Fruit	Puree	Heated 10 min
Dimethyl Sulfide	5[a]	0.4	0.0	100
Dimethyl Disulfide	13[a]	1	0.08	0.02
Dimethyl Trisulfide	1.5[1]	0.1	0.4	0.4
Methyl Thioacetate	5[b]	4	12	12
Methyl Thiobutyrate	200[b]	0.005	0.03	0.03

[a]Average of values (in water) reported by Fors(1983).
[b]Values reported (in a dairy matrix) by Cuer et al. (1979).

The concentration of dimethyl sulfide changed much more than any other compound (Fig. 5.1). Small amounts were found in the fresh fruit, but none was detected in the unheated puree. Heating caused the concentration to increase from near zero to over 500 parts per billion. Based on the average threshold value reported by Fors (1983), the aroma value of dimethyl sulfide at 500 ppb would be about 100, making it the most prominent volatile found in any of the samples.

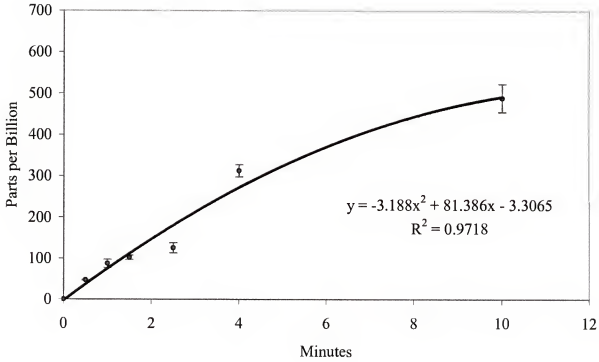


Figure 5.1. Changes in the Concentration of Dimethyl Sulfide in Strawberry Puree with Time of Heating at 95°C.

Dimethyl disulfide was found in both fresh fruit and puree. The level in the puree was nearly constant at about 0.5 ppb. The average aroma threshold of 13 ppb indicates that this compound is probably not too important to the aroma of strawberry.

Methanethiol could not be quantified because it is unstable, and it rapidly converted to other sulfur compounds such as dimethyl disulfide when added in an attempt to make a standard curve. However, its threshold in air is about 10 times lower than dimethyl sulfide, and it is very volatile, indicating that it may be important to both fresh fruit and puree. Dirinck et al. (1981) also found methanethiol to be an important aroma-active compound in strawberry. Carbon disulfide was not detected in the fresh fruit or in the unheated puree, but heating caused its concentration to increase to about 6 ppb. Unlike dimethyl sulfide, which continued to increase over the 10 minute heating time,

carbon disulfide reached its maximum after only about 1 minute and then leveled off (Figure 5.2). No threshold values (in water) were found for carbon disulfide. However, its threshold value measured in air is about 10 times higher than dimethyl sulfide, indicating that it may not contribute much to the overall aroma of heated puree.

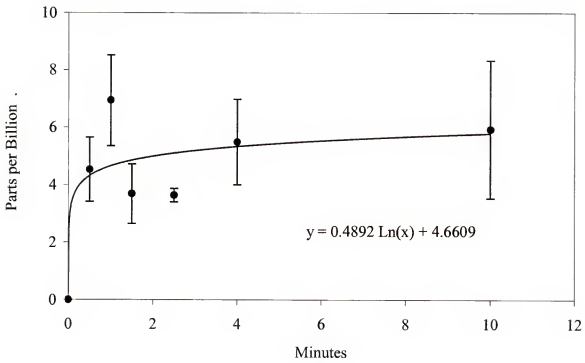


Figure 5.2. Changes in the Concentration of Carbon Disulfide in Strawberry Puree with Time of Heating at 95°C.

Two thiol esters were identified in both fresh fruit and strawberry puree. Both compounds were relatively stable during heating at a concentration of about 60 ppb for methyl thioacetate and 6 ppb for methyl thiobutyrate. Aroma thresholds for methyl thioacetate (5 ppb) and methyl thiobutyrate (200 ppb) were reported by Cuer et al. (1979) in a dairy matrix. This would indicate that of the two, methyl thioacetate (with an aroma value of 10) would be considerably more important to the aroma of strawberry than

methyl thiobutyrate (with an aroma value less than 1). Neither compound would contribute to the change in the sensory properties caused by heating.

There are a number of possible chemical pathways for the formation of sulfur volatiles. Dirinck et al. (1981) suggested that methanethiol arose enzymatically from the breakdown of methyl thioacetate and methyl thiobutyrate by thiol esterase activity. However, the fact that the concentration of both of these thiol esters did not change during this experiment, does not add support to this possibility. Sloan et al. (1969) reported that dimethyl sulfide might be formed when methionine was heated in the presence of pectin, with pectin acting as a methyl donor. They also suggested that another possible precursor could be S-methyl methionine sulfonium. In citrus, S-methylmethionine sulfonium is a precursor of dimethyl sulfide which is a major component of the off-flavor produced when citrus juice is heated (Sakamoto et al., 1996). Wong et al. (1995) evaluated the formation of dimethyl sulfide in sweet corn. They found that dimethyl sulfide production was highly correlated with S-methylmethionine sulfonium, but the role of methionine as a dimethyl sulfide precursor was not as certain. However, S-methylmethionine sulfonium is easily formed from methionine in many plants (Hattula and Granroth, 1974), and methionine may have at least an indirect role in the formation of dimethyl sulfide. Methionine also has a role in the formation of methanethiol, dimethyl disulfide and dimethyl trisulfide. Smit et al. (2000) outlined two routes of enzymatic degradation of methionine by *Lactococcus lactis* subsp. *cremoris* B78. In the first route, methionine is simultaneously deaminated and demethiolated to produce methanethiol. In the second route, methionine is converted to methional and

then to methanethiol. In both cases, methanethiol can go on to form additional sulfur compounds such as dimethyl sulfide which is a dimer of methanethiol.

It is also possible that the observed changes in sulfur compounds could be the result of binding to or release from the solid materials in the puree. In a study of sulfur compounds in broccoli, Tulio et al. (2002) added dimethyl disulfide to frozen-thawed florets. After 30 minutes at 30°C no dimethyl disulfide was detected in the headspace. The authors speculated that at the low pH of the puree, the dimethyl disulfide was loosely bound to the surfaces of the tissue disrupted by freeze-thawing. If this binding occurs in strawberry puree, the sensory impact of the sulfur compounds such as dimethyl disulfide could be much less than what would be expected based on the published threshold values of the compounds in water.

Because of the reactivity of sulfur compounds, it is possible that artifacts could be formed in the hot injector of the GC. Samples of puree were run with injection temperatures of 170°C, 200°C, and 230°C. There was no significant difference in the chromatograms obtained at the three temperatures, which agrees with the data from Boatright and Lei (2000). The authors evaluated methanethiol and dimethyl trisulfide in soy-protein isolates using GCO/MS with GC injector temperatures of 130°C and 210°C. They later compared cool, on-column injection and oven temperature of 35°C with their methods and found no evidence that the major aroma-active compounds were chromatographic artifacts formed in the hot injector.

Conclusions

The results presented here show that sulfur compounds, especially dimethyl sulfide and possibly methanethiol, can contribute to the flavor change in strawberry puree during short heating times. Although methanethiol was not quantified, its very low

aroma threshold may make it an important volatile in both fresh fruit and puree. The levels of methyl thioacetate found in fresh fruit and puree indicate that this compound may have some sensory impact, but methyl thiobutyrate probably does not.

A more complete understanding of the changes in sulfur volatiles during heating can suggest ways to reduce the formation of compounds that produce off flavors. For example, Sakamoto et al. (1996) evaluated cross-breeding of citrus to produce varieties with lower content of S-methylmethionine sulfonium which is a precursor of dimethyl sulfide. Some crosses had low levels of S-methylmethionine sulfonium which could be processed without the development of off-flavor from dimethyl sulfide. Cross-breeding may also be a useful tool for the development of strawberry varieties that form lower levels of dimethyl sulfide upon heating.

CHAPTER 6
CONCLUSIONS

Compared to batch solvent extraction and dynamic headspace concentration, solid-phase microextraction (SPME) proved to be the best method for screening strawberry samples. It is a simple, accurate, rapid, and low cost sample extraction method. Unfortunately, many aroma-active compounds could still not be quantified using SPME because they exist in concentrations below the detection limit of many common detectors. However, many of the important esters such as methyl butyrate, ethyl butyrate, methyl-2-methyl butyrate, ethyl-2-methyl butyrate, methyl hexanoate, and ethyl hexanoate could all be quantified using SPME and a FFA column. Strawberry breeding programs could benefit from the use of SPME coupled with gas chromatography to evaluate the ester content of the fruit. Esters are quantitatively and qualitatively the most important volatiles in strawberries (Latrasse, 1991). Strawberries differ markedly in their ester profile and breeding schemes crossing fruit with contrasting profiles could result in increased ester content of the fruit. Other important compounds such as diacetyl, E-2 hexenal, hexanal, linalool and γ-decalactone could also be quantified with SPME, and monitoring these compounds could also be part of a strawberry breeding program.

In general, it was found to be difficult to correlate sensory ratings and instrumental analysis. Kendall and Neilson (1966) found that a difference in concentration of 60% of geranial was required for a just noticeable difference between samples. The task of determining differences between aroma intensity between among the various compounds in the five varieties of strawberries would be a considerably more

difficult task. Most likely, the differences in the levels of aroma compounds across the 5 varieties were too small to be discerned by sensory analyses. Only the sensory ratings for peach were well correlated with the instrumental measurements, but this was largely due to the fact that the compounds most likely responsible for the peach-like aroma were not found in all varieties. Other aroma descriptors such as floral, may be too ambiguous for panelists to rate consistently, or the floral aroma may be the result of blending of more than one aroma. Flavor mixes are very complex, and even if it is known what aroma-active components are in a sample, it may not be possible to predict the sensory response. Kendall and Neilson (1966) found that a simple mixture of methyl salicylate and anethol produced a floral aroma that was uncommon to either of the two components. This blending of aromas may be one of the reasons that correlations between chemical and sensory analyses were difficult to develop.

The results presented here show that sulfur compounds, especially dimethyl sulfide and possibly methanethiol, can contribute to the flavor change in strawberry puree during short heating times. Although methanethiol was not quantified, its very low aroma threshold may make it an important volatile in both fresh fruit and puree. The levels of methyl thioacetate found in fresh fruit and puree indicate that this compound may have some sensory impact, but methyl thiobutyrate probably does not.

A more complete understanding of the changes in sulfur volatiles during heating can suggest ways to reduce the formation of compounds that produce off flavors. For example, Sakamoto et al. (1996) evaluated crossbreeding of citrus to produce varieties with lower content of S-methylmethionine sulfonium which is a precursor of dimethyl sulfide. Some crosses had low levels of S-methylmethionine sulfonium which could be

processed without the development of off-flavor from dimethyl sulfide. Crossbreeding may also be a useful tool for the development of strawberry varieties that form lower levels of dimethyl sulfide upon heating.

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BIOGRAPHICAL SKETCH

Kurt Schulbach was born in Lowville, NY, but soon moved to California. When he reached the third grade his family moved to Panama for a year and a half, and then returned to California. When he reached the sixth grade Kurt's family moved to Nicaragua for another year and a half and then returned to California where Kurt graduated from Colusa High School in Colusa, CA. Kurt continued his education and received his B.S. degree in agronomy from California State University, Chico, in 1979. Kurt then attended graduate school at Utah State University in Logan, UT, and received an M.S. in irrigation science in 1982. Kurt then worked as a cooperative extension agent specializing in irrigation and vegetables crops in Monterey County, CA, from 1983-1998. In 1996-1997 Kurt took a sabbatical to the University of Florida and studied with Dr. Brian McNeal in the Soil and Water Science Department. After returning to California, Kurt became interested in vegetable processing and decided to return to school to study food science. Kurt will graduate in 2002 with a Ph. D. in food science, specializing in flavor chemistry, and hopes to find a job in the food industry.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles A. Sims, Chair
Professor of Food Science and Human Nutrition

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Donald J. Huber
Professor of Horticultural Science

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 2002

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